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THE DETECTION OF AGGLUTININS TO *STR. AGALACTIAE* STRAINS USING A MILK RING TEST

By

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INTRODUCTION

The Abortus Bang Ring probe, or milk ring test introduced by Fleischauer (1937) and modified by many later workers (e.g. Hermann, 1939, Bruhn, 1948, and Roepke, Clausen and Walsh 1948) is now an accepted technique for detecting *Br. abortus* agglutinins in milk. The test depends on the fact that Brucella agglutinins in milk cause clumping of stained Brucella organisms, which adhere to the fat globules and rise with the cream to form a coloured layer on the surface of the milk.

During the course of studies on experimental streptococcal mastitis at this laboratory, it was decided to try to detect streptococcal agglutinins in the milk of cows experimentally infected with *Str. agalactiae* by using a ring test essentially similar to that used for detecting the Brucella agglutinins. This paper describes the use of a ring test for this purpose, and contrasts the results obtained by this test with those obtained by the whey slide agglutination test.

MATERIALS AND METHODS

Experimental animals. These were five first calf Ayrshire cows, in late lactation, experimentally infected by inoculation of *Str. agalactiae* strain S 13 into the teat sinus; the animals were housed in isolation indoors. Pre-and post-inoculation milk samples were taken.

Milk used for the ring test. Strippings samples were used in every case because their greater fat content simplified reading the test; preliminary comparative tests on fore- and strippings-milk had shown no detectable difference in agglutination titre. The titrations of test milks were done with diluent milk obtained from three cows in a herd known to be free from *Str. agalactiae* infection; the milk of these animals was consistently negative to the whey and ring tests using S 13 antigens. Fresh, raw milk was used in every case.

Preparation of the ring test antigen. The antigen was made from *Str. agalactiae* strain S 13 Lancefield type Ib (Pattison and Holman, 1953) maintained on Bactotryptose ox-blood agar plates and typed from time to time to check type-specificity. Preparation of the antigen was as follows:—

1. An 18-hour growth in Todd-Hewitt broth (Todd and Hewitt

1932) at 37°C. was centrifuged and the cells washed once in normal saline.

2. To each gram of packed cells 120 ml. of haematoxylin stain prepared as described by Roepke *et al*, were added.

3. The cells were resuspended in the stain by stirring with sterile glass rods and then heated at 60°C. for 20 minutes in a water bath. They were cooled rapidly to about room temperature.

4. After centrifugation the supernatant fluid was decanted and the packed stained cells were washed twice in 0·3% NaCl acidified to pH 3·8-4·0 with N/20 HCl. The volume of washing fluid used was 1/5th the volume of stain.

5. After washing the cells were resuspended in 0·5% phenol-saline on the basis of 15 ml. of phenol-saline to 1 g. of cells.

The colour of the prepared antigen was deep purple and the pH was 4·1. It was stable for at least two months, when kept intermittently at room temperature or at 4°C.

Technique for the ring test. Milk was tested within two hours of taking samples. The test was set up in a series of tubes containing undiluted test milk in the first tube and then twofold dilutions made in diluent milk; at the end of the series there was a control tube containing diluent milk only. Each tube contained one ml. of milk to which 0·02 ml. of stained antigen was added. After shaking to distribute the antigen evenly, the tubes were incubated at 37°C. for 45 minutes when the test was read. Degrees of positiveness were based on those given by Roepke *et al.* for the Brucella ring test.

Slide agglutination tests. The slide agglutination tests on whey were done by the method of Huddleson and Abell (1928), modified by Holman, Pattison and Gordon (1952); their strain S 13 slide antigen was used. Whey was prepared by the method given by Torrey (1929). Whey was tested the day after samples were taken.

RESULTS

The purpose of this work was to determine the efficacy of the ring test and to contrast it with the whey slide agglutination test in detecting streptococcal agglutinins in the milk of cows experimentally infected with *Str. agalactiae.*

The results of examining 220 milk samples by the two tests are given in Table I. It will be seen that there was a fairly close agreement between results for the ring test and the whey agglutination test, although it is realised that direct comparison between the degree of sensitivity of the two tests was not strictly permissible because different antigens, the sensitivity of which could be altered at will, were used.

The results given in the table indicated that the ring test antigen was slightly more sensitive on the whole than the whey agglutination test antigen, giving 29 positive results when the whey agglutination test was negative and five negative tests when the whey agglutination

TABLE I

WHEY SLIDE AGGLUTINATION TEST AND MILK RING TEST
MILK SAMPLES FROM FIVE DIFFERENT COWS INOCULATED INTO THE TEAT SINUS WITH *Str. agalactiae.* STRAIN S 13

No. of samples examined	*Slide agglutination tests on whey*		*Comparative milk ring tests*		*Whey agglutination test positive: milk ring test negative*	*Milk ring test positive: whey agglutination test negative*
	positive	*negative*	*positive*	*negative*		
220	50	170	74	146	5	29

test was positive. When the ring test was negative and the whey agglutination test positive, the whey titres obtained were low (about 1:1 or 1:2).

As one check on the specificity of the S 13 ring test antigen, milk giving a positive reaction to this antigen was tested against standard Brucella ring antigen. The result of these comparative tests showed that only the S 13 antigen reacted with the test milk.

DISCUSSION

The ring test was more easily applied in testing the milk samples than the whey slide agglutination test. Results were consistent and positive and negative reactions were distinguishable. As with the comparable Brucella ring and slide tests there was no marked difference in the sensitivity of the slide and the ring antigen when used on individual quarter milk samples. It seems likely, however, that this very crude ring antigen could be considerably improved. As one example, improvements in staining technique by giving deeper staining, would allow greater dilution of the antigen, and thus aid it to be more sensitive (Jepsen and Bruhn 1951). This might lessen the occurrence of a positive whey agglutination test with a negative ring test, a result also found by the earlier ABR workers, (e.g. Fleischauer, 1937: also Smitmanns, 1938), but which occurred much less frequently as the Brucella ring test developed (e.g. Norell and Olson, 1943; Winther and Hansen, 1943).

Results of bacteriological examination of milk samples subjected to the ring test showed that this test, like the whey plate agglutination test, detected the presence of agglutinins, but not of organisms, in milk. Several samples taken shortly after infection, when streptococci were present in the milk, were negative to the ring test, as might be expected with early infection, and many samples taken later in the disease were positive to the ring test although streptococci could not be isolated from the milk. The test was difficult to read when the milk was grossly abnormal, but this might be overcome by using a capillary test similar to that developed by King (1951) for detecting Brucella agglutinins in whole, skim, or homogenised milk.

The question of the type specificity of the S 13 ring test antigen used was not examined. All tests were made on milk from cows experimentally infected with strain S 13. However it is well known that a group-B serum will react with antigen suspensions of heterologous type strains and that the serum is not type-specific until suitably agglutinin-absorbed. If streptococcal whey agglutinins behave similarly then perhaps a ring test antigen of one Lancefield type of group-B streptococci would detect agglutinins of some other types. On the other hand Plastridge and Cunningham (1942) found that whey agglutinins were, in part at least, type specific. Numerous types were required to test the serum and the whey of milk from cows infected with *Str. agalactiae.*

SUMMARY

A milk ring test, using a haematoxylin-stained antigen of *Str. agalactiae* strain S 13 is described.

The test detected agglutinins in the milk of five cows experimentally infected with strain S 13, and was not inferior to a whey slide agglutination test for this purpose.

When compared with standard Brucella ring antigen the streptococcal antigen appeared to the species-specific.

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EFFECT OF A SUB-CLINICAL WORM-BURDEN ON THE DIGESTIVE EFFICIENCY OF SHEEP

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INTRODUCTION

Digestibility trials with sheep are frequently used to measure the feeding value of a ration. This value is determined as the percentage digestibility of the constituents of the food and is dependent for its accuracy on the digestive efficiency of the animal under observation. A number of factors may affect the digestive efficiency of sheep (Raymond, 1951) and it is important to determine their precise action. One of these factors is the size of the nematode population parasitic in the alimentary tract, particularly embarrassing at sub-clinical levels when the host betrays no external signs of parasitism.

Stewart (1933a) found a depression of the digestibility of crude protein and supported this work by the demonstration of an inhibitor of proteolytic enzyme (Stewart 1933b). Andrews (1938) and Andrews, Kauffman and Davis (1944) were unable to confirm Stewart's work but agreed that infested lambs required much more energy per pound of liveweight gain. Franklin, Gordon and Macgregor (1946) confirmed the latter work and also found a depression of C.P. digestibility. All the work quoted involved lambs with moderate to heavy infestations ; there is little indication of the number of worms necessary to produce a significant effect.

In a digestibility trial it is important that the level of internal parasitism should be less than the minimum necessary to affect significantly the digestive efficiency of the sheep used.

In a preliminary trial observations were made on twelve Suffolk x Halfbred wethers carrying a light infestation. It was concluded from this that egg-counts must be high enough to serve as an accurate index of the worm-burden, in order to show whether the latter affects digestive efficiency.

A further preliminary trial with eight pairs of twin Suffolk x Halfbred lambs, eight months old, failed to show a difference between groups but did show a clear difference between individuals in each pair of twins, presumably because of greater uniformity between the compared animals.

Egg-counts, although they provide valuable evidence of differences in the size of infestation, are subject to numerous disadvantages (Spedding, 1952). It was therefore decided to design an experiment to compare the digestive efficiency of worm-free lambs with that of their infected twins.

METHODS

Six pairs of twin lambs were raised worm-free until four months of age, by housing the ewes and lambs in indoor pens from lambing time until weaning. The pens were cleaned out every two days and the animals fed on frozen grass, hay and crushed oats containing powdered phenothiazine. At 4 months of age the weaned lambs were separated into two groups. They were numbered 3 to 14, consecutive numbers being twins, and separated so that one group (Group " O ") contained all the odd numbers and the other group (Group " E ") all the even. Thus each group contained one member of each twin pair. The lambs in Group " O " were then artificially dosed with infective larvae of *Trichostrongylus axei* at three levels of infection (Table 1), with two lambs at each level.

TABLE 1

DETAILS OF INFECTIVE DOSES

Lamb No	*Dose level*	*Number of* T. axei *larvae administered*	
		Daily	*Total*
3 & 5	1	4,000	48,200
7 & 9	2	6,000	73,000
11 & 13	3	8,000	115,800

Strongyloides papillosus was also present and the picture was therefore complicated to a certain degree by the presence of two species, one living in the 4th stomach and the other in the small intestine. However, both species are of country-wide distribution and both were present as a light infestation only, and, as the eggs are easily distinguishable from one another, the relative extent of each infestation was readily discernible.

RESULTS

The liveweight gain of the animals was studied for the ten weeks following infection, during which time the faeces of Group "O" contained increasing numbers of eggs. Group " E " faeces remained negative, except for an occasional egg of *S. papillosus*. The results of the liveweight experiment are being reported separately (Spedding 1953). It may be stated, however, that the control lambs gained weight at a significantly greater rate than the infected lambs, and that the weight gain of the latter appeared to be inversely proportional to the number of larvae administered.

The animals were then placed in digestibility crates for a preliminary period of six days, and a digestibility trial was conducted over a further sixteen day period, according to the methods of Raymond, Harris and Harker (1953). Daily dry-matter determinations of the faeces were carried out on 300 g. samples, dried

at 100°C for 24 hours. Samples for chemical analysis were taken every day, consisting of approximately 10 per cent of the day's total faecal output. These were bulked for the whole trial, for each animal, before analysis.

Egg-counting was carried out by the mixing technique recommended by Spedding (1952) and all samples were taken after the total daily faecal output had been mixed. Three counts were done on the faeces of Group " E " lambs, to confirm that they were worm-free.

A few eggs of *Strongyloides,* but none of *T. axei* occurred in the faeces of the control lambs, and they were regarded as completely worm-free for the experiment. The average e.p.d. data for Group "O" are given in Table 2. The counts are averages for the whole sixteen days based on duplicate counts each day.

TABLE 2

AVERAGE E.P.D. DATA FOR LAMBS IN GROUP O

Lamb No	*Av. E.p.d. over a 16 day period*		
	T. axei	S. papillosus	*All species*
3	529,352	291,674	821,026
5	362,335	563,240	925,575
7	131,452	346,117	477,569
9	224,542	1,495,038	1,717,580
11	403,576	1,940,153	2,343,729
13	698,325	2,989,045	3,687,370

Prior to this experiment, lamb No. 7 had lost weight rather rapidly. This trend was arrested and reversed by a single dose of 10 g. phenothiazine on 24/9/52. The egg-count of this animal was therefore much lower than would be expected at the level of infection at which it was dosed. Apart from this, there was a general correlation between egg output and dose of larvae. This was most noticeable in the total counts.

The liveweight changes are shown in Table 3. In every case the control lamb gained more weight, or lost less, than its infected twin.

The feed intake also varied considerably (Table 4). That of the infected animals was, on the average, lower than that of the controls, and, in five out of the six pairs, the infected lamb consumed less than its twin, although all animals were offered the same amount. The exceptions (Nos. 9 and 10) differed relatively little. Within the control group (E) the feed intake could not be related to the liveweight change. Within the infected group (O) there was a distinct indication that the level of feed intake decreased at the highest level of infection (dose level 3).

TABLE 3

LIVEWEIGHT PRODUCTION OF LAMBS

(17/10/52 to 14/11/52)

	Lamb No.	*Initial liveweight (lb.) 17/10/52*	*Final liveweight (lb.) 14/11/52*	*Liveweight Gain (lb.)*
"O"	3	98·7	93·6	—5·1
	5	120·9	114·4	—6·5
	7	82·5	82·7	+0·2
	9	110·8	109·2	—1·6
	11	93·7	88·2	—5·5
	13	91·5	86·6	—4·9
"E"	4	103·9	109·2	+5·3
	6	116·9	113·5	—3·4
	8	94·9	97·4	+2·5
	10	110·1	110·3	+0·2
	12	124·9	122·2	—2·7
	14	95·5	99·0	+3·5

TABLE 4

FEED INTAKE OF LAMBS

	Lamb No	*Total amnt. eaten (g. D.M.) 28/10/52 to 13/11/52*	*Av. daily consumption (g.D.M.)*
"O"	3	18,170	1,135·6
	5	18,855	1,178·5
	7	18,521	1,157·6
	9	19,033	1,189·6
	11	16,648	1,040·5
	13	16,386	1,024·1
"E"	4	19,583	1,224·0
	6	19,421	1,213·8
	8	19,365	1,210·3
	10	18,968	1,185·5
	12	19,588	1,224·3
	14	19,566	1,222·9

The digestibility data (Table 5) show that the general level of digestibility was lower in the infected than in the worm-free lambs. The digestive efficiency of the infected group appeared to be impaired, on the average, by 1.5 units of digestibility, or 2.2 per cent on a mean of 67.68 per cent. As a difference between two groups this does not appear to be very large. The standard error of the difference was 0.68, giving the 5 per cent limits for the difference as —0.27 and +3.25. The mean digestibility coefficient at each level of infection, however, exhibited a progressive decrease as the level of infection increased (Table 6). At the highest level of infection

TABLE 5

DIGESTIBILITY COEFFICIENTS AND AMOUNT OF FOOD ABSORBED

Lamb No		*Digestibility Coefficient* %	*Av. for groups*	*S.E.*	*Total amnt. of food absorbed (g. D.M.)*	*Av. amnt. of food absorbed daily (g. D.M)*	*Group Av.*	*Av. L/wt change per Lamb (lb.)*
"O"	3	68·89			12,480·4	780·0		
	5	67·23			12,676·7	792·3		
	7	66·98	66·92		12,405·1	775·3	750·7	—3·7
	9	67·31			12,810·7	800·7		
	11	66·83			11,126·6	695·4		
	13	64·47			10,564·0	660·3		
				±0·49				
"E"	4	67·47			13,213·7	825·9		
	6	68·58			13,319·8	832·5		
	8	68·48	68·44		13,260·7	828·8	830·5	—0·7
	10	69·31			13,147·4	821·7		
	12	68·55			13,428·1	839·3		
	14	68·26			13,355·0	834·7		

TABLE 6

COMPARISON OF DIGESTIBILITY AT THREE LEVELS OF INFECTION

Lamb No		*Digestibility coefft. (%)*	*Dose level*	*Mean digestibility coefft. (%)*	*Mean total E.p.d. count*
"O"	3	68·89	1	67·96	873,300
	5	67·23			
	7	67·31	2	67·15	1,097,575
	9	66·98			
	11	66·83	3	65·65	3,015,550
	13	64·47			

TABLE 7

MEAN DIFFERENCE IN DIGESTIBILITY AT EACH DOSE LEVEL BETWEEN INFECTED ANIMALS AND THEIR TWINS

Dose Level	*Mean digestibility %*		*Difference in digestibility*
	Infected	*Control*	
1	67·96	68·03	0·07
2	67·15	68·89	1·74
3	65·65	68·41	2·76

(dose level 3) the mean of 65.65 per cent represented a considerable difference from the control mean of 68.44 per cent amounting to 2.79 units, or approximately 4 per cent on the mean of 67.05 per cent.

A closer approximation to the true effect of the worm-burden is derived from a comparison of the mean digestibility of the two lambs at each dose level (Table 7). From these data it appeared that there was a depression of digestive efficiency, which exhibited a negative relationship with increasing levels of infection. A further point of considerable interest lies in the variability within each group (Table 5). Clearly the range was greater within the infected group (4.42 units) than within the worm-free group (2.08 units). This represented a range of 6.6 per cent in Group O, compared with only 3 per cent in Group E.

Chemical Analyses

Three analyses were carried out, in quadruplicate, using the methods developed by Raymond, Harris and Harker (1953). Ash determinations made possible the expression of the dry matter digestibilities on an ash-free basis. The latter are shown in Table 8, and exhibit a similar pattern to those calculated without regard to the ash content (Table 5).

The apparent crude protein digestibility shown in Table 9 indicated a difference between the groups of 3.05 units. The standard error of the difference was 1.23, giving the 5 per cent limits for the difference as —0.10 and +6.20. There was, however, no

Table 8

Apparent Digestibility Data on an Ash-free d.m. Basis

Lamb No		*Per cent app. digestibility coefft. (Ash-free D.M.)*	*Per cent mean coefft. of digestibility at each dose level for each group*		*S.E. of group means*
"O"	3	71·49	70·96		
	5	70·44			
	7	69·53	69·96	69·88	
	9	70·38			
	11	69·73	68·71		
	13	67·69			
"E"	4	70·78	71·32		±0·38
	6	71·85			
	8	71·51	71·99	71·52	
	10	72·47			
	12	71·48	71·26		
	14	71·03			

definite relationship within the infected group between size of infective dose and crude protein digestibility.

No significant effect was observed on the crude fibre digestibility coefficients, shown in Table 10. The feed analysis on which these figures are based is given in Table 11. These analyses were carried out in quadruplicate on oven-dried samples of the grass, taken when cut and mixed.

TABLE 9

APPARENT C.P. DIGESTIBILITY DATA

Lamb	*C.P. digestibility coefft. %*	*Coeffts at each dose level*	*Group means*	*S.E. of group means*	*Differences between pairs of twins at each level*
3	66·01	63·59			2·09
5	61·17				
7	63·54	64·43	62·98		2·18
9	65·32				
11	65·02	60·91			4·88
13	56·80				
				±0·87	
4	64·89	65·68			
6	66·48				
8	65·58	66·61	66·03		
10	67·64				
12	67·23	65·79			
14	64·36				

TABLE 10

APPARENT C.F. DIGESTIBILITY DATA

Lamb No	*C.F. digestibility coefft. %*	*Coefft. at each dose level*	*Group means*	*S.E. of group means*	*Differences at each dose level between pairs of twins*
3	74·59	74·15			+0·23
5	73·70				
7	73·64	74·49	74·02		—1·63
9	75·34				
11	74·25	73·41		±0·45	—0·69
13	72·57				
4	73·47	73·92			
6	74·37				
8	75·39	75·85	74·62		
10	76·30				
12	72·97	74·10			
14	75·22				

TABLE 11

FEED ANALYSIS :
CHEMICAL COMPOSITION OF THE HERBAGE FED, ON A D.M. BASIS

Ash %	*C.P.* %	*C.F.* %
10·05	16·12	22·8

DISCUSSION

The depression of the apparent digestibility of crude protein is in agreement with the work of Stewart (1933a) and Franklin *et al* (1946), and disagrees with the results obtained by Andrews (1938, 1939) and Andrews *et al* (1944). The species of worm used should, however, be taken into account. Stewart (1933) employed animals carrying a mixed infestation. Andrews (1938, 1939) used *Cooperia curticei;* Andrews *et al* (1944) and Franklin *et al* (1946) used *T. colubriformis*. In the present work it is important to note that the experimental animals had previously exhibited an inverse relationship between liveweight gain and size of infestation. Thus a fairly coherent picture of the worm-damage emerged. This suggests a decreased rate of liveweight gain due chiefly to a considerable reduction in appetite and to a reduction of digestive efficiency. The latter term is used to indicate an impaired digestive economy and not necessarily any interference with the strict process of digestion. The apparent digestibility coefficient is a measure of the amount of feed absorbed. Thus crude protein could be digested by a sheep but not absorbed, due, possibly, to worm damage to the absorptive tissues. The nitrogen excreted would however, be calculated as crude protein and give a lowered apparent digestibility coefficient for this fraction. This would be further exaggerated by any increase in the metabolic nitrogen due to the activity of the parasites. The importance of this is that a lowered digestive efficiency does not necessarily support the contention that nematodes secrete an anti-enzyme.

The closest approach to the present work appears to have been that of Franklin *et al* (1946). Two differences should however be noted. First, in the present experiment grass was used as the feed. The results may be expected, therefore, to simulate more closely those from normal pasture fed sheep. Secondly, the infestation used appears to be at a lower level.

The lack of effect on the digestibility coefficients for crude fibre is in agreement with earlier workers, although Stewart (1933) considered that there were indications of increased absorption of crude fibre as the degree of nematode infestation decreased. Unfortunately

Stewart's work depended upon egg-counts and there were no worm-free controls. The same author suggested that if nematode infestation does, in fact, inhibit the digestion of crude fibre, this would indicate a certain amount of fibre digestion in the small intestine.

The depression of appetite in infected lambs is in complete accord with Gibson (1951), who regarded this as probably the major factor of damage due to *T. axei*. The appetite depression was about 8 per cent compared with the feed intake of the control lambs. The depression of the digestibility coefficients, however, means that the infected lambs absorbed 10 per cent less than the controls, due in part to a lowered intake and in part to a lowered digestive efficiency.

Two relevant points must be considered in connection with feed intake. The lowered intake might affect digestibility in two ways, namely (a) directly, and (b) by allowing the animals to "select" i.e. to eat a non-representative sample of the feed offered by selecting the more attractive parts. "Selection" usually involves the ingestion of the more nutritious portions e.g. "leaf" as against "stem". In consequence, it operates to raise digestibility. The evidence bearing on the direct effect of level of feed intake on digestibility indicates that animals eating less may digest more efficiently (Raymond 1952). Both of these factors, therefore, would operate to raise the digestibility of the foods given to the lambs with the lower intake, that is, in an opposite direction to the differences found.

The implications of these results are twofold. First, that the damage due to sub-clinical levels of worm-infestation involves both a depression of appetite and a depression of digestive efficiency. These factors limit the potential liveweight gain of sheep, whatever the quality of feed offered. Secondly, that in experimental nutritional work some interference may be expected where noticeable differences in worm-burden exist between experimental animals.

The number of worms that must be present before this interference becomes measurable would vary with the species of worm and, probably, with each individual host. It would be advantageous if this level of infestation could be defined in terms of egg-counts, so that animals with higher counts could be rejected by the experimenter or be dosed with anthelmintics. The best indications from the present work were at dose level 1. The digestive efficiency was not markedly impaired and previous liveweight gain had been only slightly less than that of worm-free lambs. This level, then, was one at which the adverse effects were just measurable. It is best defined by considering (a) the number of infective larvae adminstered (48,200) and (b) the average egg-output over its peak period. The latter was approximately 500 *T. axei* e.p.g. (500,000 e.p.d) and 300 *S. papillosus* e.p.g. (300,000 e.p.d.). Counts of this order have not previously been regarded as serious in lambs, but it would seem that they must be below this if adverse effects are to be eliminated.

SUMMARY

The effect of a sub-clinical worm-burden on the digestive economy of sheep has been investigated by digestibility trials on shearlings, twin lambs, and lambs artificially infected with *Trichostrongylus axei* compared with worm-free controls.

A significant depression of the dry-matter digestibility coefficients was found in the last experiment. The apparent digestibility of crude protein was significantly lowered in the infected lambs, while the crude fibre fraction appeared to be unaffected.

The infected lambs also exhibited an 8 per cent depression of appetite. With the lowered digestive efficiency, this meant that an infected animal absorbed 10 per cent less food than a worm-free lamb.

It is suggested that these two factors are the chief means whereby worms depress the liveweight gain of the host.

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THE EFFECT OF CHEMOTHERAPEUTIC AGENTS ON *MYCOBACTERIUM JOHNEI*

By

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INTRODUCTION

Several clinical reports have been published on the treatment of Johne's disease in cattle with streptomycin (Larsen, Vardaman and Groth, 1950; Cauchy, Goret, Mérieux and Verge, 1951; Ford, 1952), sulphones (Verge, Goret and Cauchy, 1951; Larsen and Vardaman, 1952), para-amino-salicylic acid (Oestenbrug and Talsma, 1951), para-acetamido-benzaldehyde thiosemicarbazone (Ford, 1952), and isonicotinic acid hydrazide (Larsen and Vardaman, 1953). In no case was treatment successful.

Because of the lack of suitable culture media very few determinations have been made *in vitro* of the sensitivity of *Mycobacterium johnei* to chemotherapeutic agents. Johnson (1947) found that streptomycin, 14μg./ml., prevented growth of a culture of *M. johnei*. Larsen *et al* (1950) and Larsen and Vardaman (1952) also working with one culture, which was able to grow in media that did not contain the *M. phlei*. growth factor, found it to be sensitive to 2μg./ml of streptomycin and to 6μg./ml. of viomycin and to be resistant to promin, stilbamidine, pentamidine isethionate, 4-aminosalicylic acid, 4:4^1-diamino-diphenyl-sulphone and four thiosemicarbazones. Larsen and Vardaman (1953) found that 25μg,/ml, of isonicotinic acid hydrazide was necessary to inhibit the growth of a culture of *M. johnei* they examined.

It seemed worthwhile to study the effect of therapeutic agents on a considerable number of recently isolated strains of *M. johnei* that had not been adapted to grow in media lacking the *phlei* factor. This work was made possible by modifying Dubos's (1945) liquid medium, a medium very suitable for determining *in vitro* the sensitivity of *M. tuberculosis* to chemotherapeutic agents, to enable it to support the growth of *M. johnei* (Williams Smith, 1953).

MATERIALS AND METHODS

Cultures. Ten strains of *M. johnei* isolated from cases of Johne's disease were studied; nine were from cattle and one from sheep. None of them would grow in media to which *phlei* factor had not been added. The classical human strain of *M. tuberculosis,* H37Rv, whose sensitivity to chemotherapeutic agents has been widely studied, was included in this investigation as a control.

Chemotherapeutic agents. The following substances were studied; streptomycin, sodium para-aminosalicylate, isonicotinic acid hydrazide, sulphetrone, 4:4[1] diamino-diphenyl-sulphone (D.D.S.), sulphathiazole, penicillin, chloramphenicol and terramycin. They were used as freshly prepared aqueous solutions with the exception of D.D.S. which, on account of its low solubility, was dissolved in the medium used for carrying out the sensitivity tests.

Culture medium. The liquid medium that was used for all the sensitivity tests has been described fully elsewhere (Williams Smith, 1953). Its formula was as follows :—

Potassium dihydrogen phoshate, KH_2PO_4	1·0g
Disodium hydrogen phosphate $Na_2HPO_4 \cdot 12H_2O$	6·25g.
Sodium citrate	1·5g
Magnesium sulphate $MgSO_4 \cdot 7H_2O$ ..	0·6g.
Tween 80	0·5ml.
Bacto-casitone (Difco)	2·0g.
Alcoholic extract of *M.phlei*	20·0ml*
Bovine serum	200·0ml.
Glass distilled water	to 1,000·0ml.

* Equivalent of 5 g. of dried *M.phlei.*

This medium was contained in screw-capped bottles in 2·5 ml. amounts.

Method of carrying-out tests. Series of two-fold falling dilutions of each chemotherapeutic agent were made in the bottles of medium giving a final volume of 3·0 ml. in each bottle. Eleven series of dilutions of each chemotherapeutic agent were made so that the ten strains of *M. johnei* and the *M. tuberculosis* strain, H37Rv, could be studied at the same time. Each series, except those containing sulphonamides, was inoculated with 0·02 ml. of one of the cultures growing in the liquid medium; the sulphonamide series were inoculated with 0·002 ml. Incubation was then carried out at 37°C. Observations were made at weekly intervals for 25 days when final readings were made.

RESULTS

The sensitivity of the ten strains of *M. johnei* and the strain of *M. tuberculosis,* H37Rv, to streptomycin, sodium para-aminosalicylate, isonicotinic acid hydrazide, sulphetrone, 4:4[1] diamino-diphenyl-sulphone (D.D.S.), sulphathiazole, penicillin, chloramphenicol and terramycin is shown in Table 1. The *M. johnei* strains and H37Rv were very sensitive to streptomycin, 0·25 to 2·0μg./ml. being sufficient to inhibit bacterial growth. As much as 50 to 200μg./ml. of isonicotinic acid hydrazide were required to prevent growth of the *M. johnei* strains although H37Rv was inhibited by 0·3μg./ml. The *M. johnei* strains showed a somewhat similar degree of resistance to terramycin as H37Rv, 20 to 80μg/ml. being required for inhibition.

TABLE I

THE IN VITRO EFFECT OF CHEMOTHERAPEUTIC AGENTS ON *M. Johnei*

Drug	*Minimal inhibiting concentration (μg/ml.) for strain number*										
	1	2	3	4	5	6	7	8	9	10	*M. tuberculosis H37Rv.*
Streptomycin	0·25	1·0	1·0	1·0	2·0	1·0	0·5	1·0	1·0	0·5	1·0
Isonicotinic acid hydrazide	100	50	200	50	100	>200	50	50	100	50	0·3
Sodium para-aminosalicylate ..	>200	200	>200	>200	100	>200	100	>200	>200	>200	0·6
Sulphathiazole	>500	>500	>500	500	>500	>500	>500	500	500	500	25
Sulphetrone	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	200
4·4[1] diamino diphenyl-sulphone (D.D.S.)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	50
Penicillin*	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
Chloramphenicol ..	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	20
Terramycin	20	40	>80	80	20	20	40	80	40	20	20

* The concentrations of penicillin are expressed as units /ml. not μg/ml.
>=greater than

With a few exceptions all the *M. johnei* strains grew in the highest concentrations of all the other agents tested although some slight decrease of growth rate was occasionally noted in the higher concentrations. In contrast the growth of H37Rv was completely inhibited by 0·6μg./ml. of sodium para-aminosalicylate, 25μg./ml. sulphathiazole, 200μg./ml. of sulphetrone, 50μg./ml. of D.D.S., and 20μg./ml. of chloramphenicol; like the *M. johnei* strains, however, it grew well in media containing 200 units/ml. of penicillin.

DISCUSSION

In view of the difficulties encountered in other methods of controlling Johne's disease the use of chemotherapeutic agents is at least worthy of consideration. The present work has shown that *M. johnei* is much more resistant to the action of these agents than is *M. tuberculosis* but all ten cultures were sensitive to streptomycin. Although published work (*loc. cit*) has shown this agent to be valueless in the treatment of clinical cases of Johne's disease it could be argued that those cases were too advanced and in all probability large areas of the intestinal tract had been irreparably damaged before treatment was commenced. It is possible that quite different results would be obtained if early cases were treated, the diagnosis of which might be facilitated by the use of the complement-fixation test (Hole, 1952). It is as well, however, to consider the position existing in the treatment of tuberculosis in human beings. The treatment of this disease with streptomycin requires its continuous daily administration for 3 to 6 months and best results are obtained if other agents such as para-aminosalicylicacid or isonicotinic acid hydrazide are given at the same time, as this combined therapy tends to suppress the development of drug-resistant variants. One reason for the necessity of prolonged treatment is the fact that streptomycin (like many other agents) is only active on bacterial cells in the process of division and the rate of division of *M. tuberculosis* is slow. The rate of division of *M. johnei,* at least in vitro, is even slower and none of the agents used to suppress the development of resistant variants in the treatment of tuberculosis are active against *M. johnei.* It is improbable, therefore, that the chemotherapeutic agents available at present would have any practical application in the treatment of Johne's disease.

SUMMARY

The sensitivity of ten recently-isolated strains of *M. johnei* and the H37Rv strain of *M. tuberculosis* to nine chemotherapeutic agents has been studied in vitro using a modification of Dubos's liquid medium.

Generally speaking, the *M. johnei* strains were more resistant to the chemotherapeutic agents than was H37Rv. They were quite sensitive to streptomycin but were resistant to isonicotinic acid

hydrazide, sodium para-aminosalicylate, sulphathiazole, sulphetrone, 4:4[1] diamino-diphenyl-sulphone, penicillin, chloramphenicol and terramycin.

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SOME OBSERVATIONS ON GENERAL ANAESTHESIA IN PONIES

By

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INTRODUCTION

In recent years a variety of new ways of anaesthetising horses have been described; for example Marnac and Lemetayer (1930) reviewed earlier work and described a technique for the use of chloral hydrate, Henkels (1938) devised an apparatus for the administration of ether by inhalation, Francis and Parry (1949) introduced β naphthoxy-ethanol, a new anaesthetic drug, and in 1952 Dyce, Jones and Wadsworth described a method employing cyclopropane. This search for new techniques and new drugs illustrates the difficulties associated with general anaesthesia in the horse.

During the development of techniques for the fistulation of the horse's intestine (Alexander and Donald, 1949; Alexander, 1951, 1952) experience was obtained in anaesthetising ponies for periods of up to three hours. Since opportunities of observing the effects of anaesthesia of this duration are unlikely to be of frequent occurrence in general practice, it was felt that the description of a method of anaesthetisation with pentobarbitone which had proved satisfactory, together with some observations on the use of chloral hydrate and ether for long anaesthesias, would be of interest.

METHODS

Pentobarbitone. A solution of sodium pentobarbitone containing 60 mg/ml (Abbotts Veterinary Nembutal) was given by injection into the jugular vein. Anaesthesia was induced with the animal in a standing position without restraint other than an assistant holding the head. The induction dose was computed on the basis of 20 ml solution per 50 kg and an appropriate number of 20 ml syringes were prepared. The first 20 ml was injected rapidly and the syringe changed; the anaesthetic solution was then injected slowly until the animal subsided to the ground. In animals of 100 to 250 kg. recumbency was produced by between 25 to 40 ml of solution. With the animal lying quietly on the ground the injection of anaesthetic solution was continued until full anaesthesia had been induced.

As the operation progressed, additional doses of pentobarbitone were given. This was facilitated by using an indwelling cannula. The indication for the administration of these additional doses was determined by the animal's response to the operation, the anaes-

TABLE I

PENTOBARBITONE SODIUM ANAESTHESIA IN PONIES

DOSE IN MG/KG

Pony	*Weight* kgs	*Induction*	*Maintenance* *Time in Minutes after Induction*													
			10	20	30	40	50	60	80	100	120	140	160	180	200	210
Ginger Op 1	222	14·4														
Ginger Op 2	222	15·0		3·0		3·0		2·4			1·8					
Ginger Foal Op 1	138	14·5	4·8		4·8		4·8									
Ginger Foal Op 2	138	14·5	4·8		4·8		3·4		3·4		4·8					
Black Foal	129	20·7														
Judy Op 1	102	19·6	3·3			6·5			6·5			5·2			6·5	
Judy Op 2	154·5	9·5	2·0	2·0	6·0		2·0	4·0			4·0					
Rufus	204	13·1														
Tilly Op 1	167	11·9														
Tilly Op 2	167	17·9	2·0	1·0		1·0			1·5		1·0					
Tilly Op 3	198	10·1	3·4	3·4			2·5									
Blackie Op 1	191	24·4	3·5	3·5		3·5										
Blackie Op 2	191	13·9														
Blackie Op 3	176	10·5		3·0			3·0			3·0	1·5	1·5	1·5		1·5	1·5
Punch Op 1	131	20·4	5·1	5·1		3·7	2·5									
Punch Op 2	131	13·2	2·0	5·0	4·0	3·5	3·5	3·5			3·5		3·5			

thestic solution being injected slowly until the required depth of anaesthesia was produced.

The respiratory depression produced by anaesthetic doses of pentobarbitone produced cyanosis of the tissues which it was found advantageous to treat by the continuous administration of oxygen with 5 per cent. carbon-dioxide. This was done by passing a catheter into the nasal cavity, connecting it to a cylinder containing the mixture of gases and allowing a gentle flow of gases to pass into the nostrils. Table I shows the quantities of pentobarbitone required to induce and maintain anaesthesia in sixteen cases.

Chloral hydrate. The drug was dissolved in water to make a 10 per cent solution. The initial dose was calculated on the basis of 7 g. chloral hydrate per cwt. body weight and additional injections of 100 ml of the solution were given at intervals to maintain the required depth of anaesthesia.

Thiopentone/Ether Anaesthesia. A 10 per cent solution of thiopentone was injected intravenously until recumbency was produced. This required a dose of 20 to 25 mg/kg. With the animal recumbent the anaesthetic apparatus of Henkels (1938) was fitted and the administration of ether started. The rate of administration of ether was adjusted so as to maintain the required depth of anaesthesia.

Table II

Deaths Probably Due to Anaesthetic Poisoning

	Chloralhydrate	*Thiopentone/Ether*	*Pentobarbitone*
Number of Anaesthesias ..	4	4	20
Number of Deaths ..	2	2	0

RESULTS

Table II shows the deaths which occurred during or following 28 consecutive anaesthesias. Extracts from the post-mortem reports by Mr. I. S. Beattie of the four anaesthetic deaths are detailed below. The deaths appeared to be attributable to the anaesthetic drug.

Deaths After Chloral Hydrate

No. 146/149. A 5 year old Dartmoor Pony Mare (250 kg) which received a total of 60g chloral hydrate and was under the anaesthetic for three hours died 4 days after the operation. Petechiae were present on the pleura, and the lungs were congested and oedematous. The myocardium showed fatty change. The liver was deep yellow with dark brown mottling, soft and friable. The kidneys were pale, soft and the cortical tubules showed diffuse fatty change. The operation wound was healing normally. Death was probably due to chloral hydrate poisoning.

No. 358/48. An aged Pit Pony Mare which was operated on the 11th November, 1948 and appeared to be progressing well, died suddenly 7 days after the operation. At post-mortem the carcase was in good condition. On opening the abdominal cavity a small quantity of yellowish, turbid, serous fluid escaped and multiple congested tags of an organising peritonitis were observed scattered over the visceral and parietal peritoneum, these lesions becoming confluent and more prominent in the region of the operation wound in the left flank. A large verminous aneurysm with extensive thrombosis involved the cranial mesenteric artery; both kidneys showed infarcts of various sizes probably arising from this thrombosis The lungs were slightly congested and also presented a number of small infarcts. The myocardium was pale and fatty with a single. infarct in the wall of the left ventricle. The liver was pale and mottled.

Deaths after Thiopentone/Ether:

No. 63/50. A 7 year old pony mare which was free from clinical signs of disease. A fistula of the colon was prepared which required 6g. Thiopentone to induce, and 1000 ml. ether to maintain, anaesthesia. The operation lasted approximately 2 hours. For three days following the operation the animal was dull and not eating and jaundice appeared on the fourth day. Eight days after the operation the animal became prostrate and was shot. Post-mortem examination showed that the operation wound was healing normally. The lungs were congested and oedematous. The liver, kidneys and heart showed widespread and advanced fatty change probably associated with the toxic action of the anaesthetics.

The second of these deaths was that of a 4 year old Dartmoor pony in good condition with no clinical evidence of disease. Anaesthesia was induced with Thiopentone and continued with ether. There was no undue struggling or excitement. The projected laparatomy was through an incision along the linea alba; however, as soon as the peritoneum was incised the animal collapsed, heart and respirations failing together. No post-mortem examination was made.

DISCUSSION

Marcenac, Carnus and Comby (1935) claimed to have anaesthetised about five-hundred horses with intravenous injections of chloral hydrate without observing any ill-effects. Wright (1952) recorded a single fatality in 300 cases in which chloral hydrate was used as narcotic or anaesthetic. A similar claim has been made for inhalation anaesthesia with ether, Henkels (1938) having anaesthetised five hundred horses in this manner with a complete absence of complications. In view of these claims it was surprising to find these drugs causing deaths in the small number of cases recorded here. The

explanation of this difference was not obvious; it might be a question of total amount of drug used, since most veterinary operations are of short duration whereas the anaesthesias described here lasted up to 3 hours; however, Marcenac and Lemetayer (1930) mentioned operations of 3 to 4 hours duration being conducted under chloral hydrate anaesthesia. The deaths recorded here from chloral hydrate and ether anaesthesia were not due to these drugs being used for the earliest operations in the series and, therefore, due to inexperience, as all three drugs were used with equal frequency in the first twelve operations. It then seemed likely that pentobarbitone had advantages not possessed by the other drugs both of which by this time had been responsible for fatalities.

The toxic effect of chloral hydrate on the myocardium and liver is mentioned by Wright (1952); therefore, the lesions found after anaesthesia with this drug were not unexpected. Although the death of the second pony (146/49) was probably due, in part, to the numerous infarcts, the extensive damage to the liver and heart was probably caused by chloral hydrate and must have been a contributory factor.

Henkels (1938) in 500 horses given ether by inhalation and Longley (1950) in six given ether intravenously encountered no signs of toxicity; it was, therefore, surprising to find a death after ether anaesthesia in which there were extensive pathological changes in the heart and liver. The death which occurred in the early stages of an operation under ether anaesthesia was difficult to explain. In addition to the toxic properties of ether, a further disadvantage of the Henkels apparatus was the unpleasant atmosphere produced by large volumes of ether vapour being blown through the expiratory valves into the theatre. This objection would be reduced when operating in the open.

Although pentobarbitone seemed a safer anaesthetic in ponies than either chloral hydrate or thiopentone/ether, it was not free from objection. The main disadvantage was the length of time taken by the animal to regain equilibrium. After an operation of two hours duration, the ponies were not usually capable of standing until about three hours after the last dose of anaesthetic. The animals appeared conscious before this time but did not regain control of their hind limbs. This objection was equally applicable to chloral hydrate. Excitement during the induction of anaesthesia with pentobarbitone was not observed; however, during the period of recovery there was some struggling especially when the animals attempted to rise. These movements were not difficult to control in ponies but might be a serious objection to the use of this anaesthetic in larger animals.

The respiratory depression and consequent anoxia produced by pentobarbitone was alleviated by the intra-nasal administration of oxygen with 5 per cent carbon-dioxide. However, a further disadvantage common to both chloral hydrate and pentobarbitone when used as general anaesthetics was the lack of muscular relaxation. In laparotomies through the flank this disadvantage was overcome

by blocking the last six intercostal nerves with a 1/1000 solution of cinchocaine.

It was interesting to note that the dose of pentobarbitone required to induce anaesthesia in ponies was about half that used in dogs, (Wright 1952).

SUMMARY

Twenty-eight laparotomies on ponies have been carried out under general anaesthesia. Sodium pentobarbitone appeared to be a safer anaesthetic for major surgical procedures than either intravenous chloral hydrate or the combination of sodium thiopentone intravenously followed by the inhalation of either.

The technique of anaesthetisation with sodium pentobarbitone has been described.

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JOHNE'S DISEASE IN SHEEP

By

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INTRODUCTION

It is common experience amongst sheep flocks in the S.E. Border area of Scotland for a number of ewes to be lost every year from a wasting disease. The animals lose condition over a period varying from a few weeks to several months, the wool becomes loose and severe emaciation develops. Diarrhoea is usually not present. The worm egg count varies from negligible to considerable numbers but even after adequate anthelmintic treatment no improvement occurs, nor does regular dosing with cobalt have any effect. A number of such sheep have been examined over the last five years.

MATERIAL AND METHODS

Material obtained from fifty-one sheep since the beginning of 1951 has been examined in detail. In addition material from a considerable number of sheep obtained prior to 1951 has been examined by histological methods only. The number of cases quoted in the following paragraphs refer to those seen since 1951 but the figures given cannot be taken to represent the incidence of the various types.

Tissues for histological examination were fixed in a mixture of 9 parts saturated aqueous mercuric chloride with 1 part of commercial formalin, and consisted of portions of abomasum, small intestine, colon, rectum, mesenteric lymph glands, liver, spleen, lungs and kidney. Sections were stained with haematoxylin and eosin, Giemsa, Ziehl-Neelsen and reticulin silver stains.

For bacteriological examination two methods were used. Smears were made and stained by the Ziehl-Neelsen method from mesenteric lymph glands and the mucosa of the bowel at various levels. Many smears were examined in those cases in which *Mycobacterium johnei* could not be demonstrated in the original ones. In addition, a number of smears were stained with Giemsa when acid-fast bacteria could not be demonstrated.

Tissue from various areas of intestinal mucosa and from mesenteric lymph glands was treated with oxalic acid as recommended by Taylor (1950). Since the medium prepared by Taylor (1950), which is a modification of Finlayson's (1946) medium, had been previously shown to be capable of growing the three types of johne bacilli found in sheep (Taylor 1951), it was decided to use this medium in the present investigation. Six bottles of medium were

*With the technical assistance of R. B. Cockburn.

thickly sown with each oxalic acid-treated preparation, incubated at 37°C., and examined weekly for signs of growth.

The present paper is concerned mainly with the histological findings; the bacteriological examinations are not yet complete and will be given more fully in a later paper. Representative strains have been examined biologically to eliminate the possibility of these organisms being *Mycobacterium tuberculosis*.

RESULTS

It was found that the chronic enteritis observed in the present series of cases could, for convenience of description, be divided by means of microscopic lesions into the following four groups. There is no sharp distinction between one group and another, and in addition the lesions of groups 3 and 4 are often seen together in the same bowel.

Group I (eight cases)

Post-mortem examination. At autopsy the carcase is very emaciated with excess fluid in the pleural and peritoneal cavities and replacement of fat depots with myxomatous connective tissue. The small intestine may be distinctly thickened especially the ileum and terminal portions of the jejunum, and may feel doughy to the touch. The mucous membrane of the thickened portions is distinctly granular and may be congested. It is amongst this group (7 cases) that the mucous membrane frequently has a bright yellow colour which might be confused with bile staining. The associated lymph glands are not greatly enlarged but are oedematous when sectioned. The lymphatic vessels are usually not obvious but an occasional case will show twisted lymphatic cords running over the surface of the bowel.

Histological examination. The normal mucous membrane is replaced by epithelioid cells to a varying extent. The lesions would appear to be focal in origin but quickly progress until the normal structures of the lamina propria are replaced by masses of epithelioid cells which gradually eliminate the crypts of Lieberkuhn and distend the villi (Fig. 1). In some cases this cellular reaction is confined to the mucous membrane but in others the cellular masses invade through the muscularis mucosae and replace the submucous structures (Fig. 2). No matter what degree the macrophage proliferation has reached there is no evidence of necrosis, caseation or giant cell proliferation. In advanced lesions practically the whole mucosa and submucosa are replaced by epithelioid cells, only a few remnants of glands and muscularis mucosae remaining. A marked feature of this type of lesion is the complete or almost complete absence of cell types other than the epithelioid cell. The lamina propria of the normal bowel which fills the spaces between the glands and forms the core of the

villi, consists of an argyrophil framework with variable numbers of lymphocytes, plasma cells, granular leukocytes, especially eosinophil leukocytes, and other cell types. In the advanced lesions of Johne's disease just described these cells are eliminated and only epithelioid cell masses remain. In the areas of less advanced focal lesions the cellular appearance of the lamina propria remains normal around the expanding islands of epithelioid cells without any leucocytic infiltration or fibroblastic proliferation. These early focal lesions are not immediately beneath the surface epithelium but arise well within the lamina propria. In the majority of cases there appears to be little or no involvement of the lymphatic vessels of the intestine nor is the peritoneal surface affected. Examination of the lymph glands shows no specific lesion. The subcapsular space contains a number of epithelioid cell groups or a general epithelioid cell infiltration while the macrophages within the medullary sinuses increase in number. The afferent lymphatic vessels frequently appear filled with epithelioid cell groups.

Bacteriological examination. Smears of both the intestine and mesenteric lymph glands stained by the Ziehl Neelsen method show very large numbers of *M. johnei.* Sections of these tissues stained by the same method also show very large numbers of intracellular johne bacilli wherever epithelioid cells have accumulated.

The intense yellow colour of the intestine suggests that the organism is of the pigmented variety and cultural examination as far as it has been successful confirms this.

Group II (16 cases)

Post-mortem examination. At autopsy the findings may be very similar to those described in Group I except that the mucous membrane of the intestine has not the yellow colour seen in the previous group. It must be stressed that frequently the lesions in the intestine are very slight and the thickening and granularity of the mucous membrane can only be recognised after considerable experience of the normal bowel. The terminal portion of the small intestine usually shows the greatest degree of abnormality but occasionally the jejunum may show the more obvious change. The regional mesenteric lymph glands may also be enlarged and very oedematous when sectioned. The mesenteric lymphatic vessels are occasionally distended and prominent.

Histological examination. The histology is essentially similar to that described in Group I in that the lesion consists of epithelioid cell proliferation or infiltration. The extent and distribution of the lesion, however, varies considerably from case to case and even from section to section in any one case. The epithelioid cell proliferation may be very considerable so that it destroys by pressure atrophy many of the glands of the small intestine (Fig. 3). In other sections the epithelioid cell proliferation is very much less extensive, areas of

epithelioid cells being confined to the lamina propria immediately below the surface epithelium of a villus and not extending deep into the lamina propria, so that there is little or no destruction of glands. Such lesions occur only here and there amongst normal tissue in any one section. In other areas there may be very large numbers of small foci of epithelioid cells, each focus consisting merely of a few cells which form into small microscopic nodules. Such nodules may be isolated in the lamina propria (Fig. 4) or on the other hand a considerable number may occur together to occupy areas of similar size to the more limited areas of epithelioid cell proliferation (Figs. 5, 6) but the cells have a nodular and not a diffuse distribution. In all cases of Group II the leucocytic content of the lamina propria surrounding the epithelioid cell proliferations is very considerable and at least so far as the focal areas are concerned the impression is gained that the foci of epithelioid cells are definitely being confined by the leucocytes of the lamina propria although the cells are not in inflammatory zones. The involvement of the submucous layer varies very considerably but in no case examined has the infiltration been nearly so extensive as in Group I. The usual finding is isolated groups of epithelioid cells scattered within the connective tissue or within lymphatic channels. In addition, around these follicles there is an extensive infiltration of lymphocytes and other inflammatory cells. This infiltration quite frequently occurs even where no epithelioid cells can be found but there can be little doubt that the cellular reaction is part of the johne lesion. The muscular coats are usually normal but the peritoneal surface of the intestine may be infiltrated with inflammatory cells and epithelioid cell groups. The latter may be progressing to necrosis or show changes suggesting hyalinization or fibrosis. The afferent lymphatic vessels seen leaving the serosal surface of the affected bowel and reaching the mesenteric lymph glands may show all stages of inflammation from epithelioid cell groups in the lumina to extensive necrosis, caseation and fibrosis. The mesenteric lymph glands also show varying degrees of involvement. The subcapsular sinuses are frequently packed with epithelioid cells while throughout the lymphoid nodules epithelioid groups with occasional giant cells are frequent. On occasion the whole of the lymphocyte-producing areas are replaced by confluent epithelioid cell foci and the medullary sinuses are packed with macrophage or epithelioid cell types.

Bacteriological examination. M. johnei can readily be demonstrated in smears made from the mucous membrane of the intestine or from the lymph glands. The number of bacilli are, however, not nearly so numerous as in Group I and not infrequently several smears may have to be made for a positive diagnosis. In sections the bacilli can readily be seen in the larger areas of epithelioid cell infiltration but often only in comparatively small numbers. In the smaller foci which consist of only a few epithelioid cells and which tend to remain

restricted no organisms can be demonstrated. As described above these epithelioid nodules may occur in groups so that bacilli may be absent in a considerable cellular infiltration. Similarly, while it is often difficult to demonstrate bacilli in the submucous lesions, considerable numbers of *M. johnei* can be seen in the lymphatic vessels and in the mesenteric lymph glands.

Colonies of acid alcohol fast bacilli resembling *M. johnei* were cultured on Finlayson's medium from cases in the group. Pigmentation was absent.

Group III (21 cases)

Post-mortem examination. At autopsy the findings may be very similar to those described in Group II, but there is often a greater difficulty in appreciating the thickening of the bowel. A common finding is a cracking of the mucous membrane when the bowel is bent over the fingers. In many cases, however, apart from a slightly thickened and fleshy appearance no abnormality would be suspected. The mesenteric lymph glands especially those associated with the terminal portion of the small intestine are in all cases considerably enlarged in size and obviously project the overlying mesentery into distinct bulges. The cut surface is oedematous while occasional small white flecks can be seen scattered throughout the area of lymphoid follicles.

Histological examination. The mucous membrane of the thickened portion of the bowel on superficial examination shows a definite increase in the lamina propria along with partial or complete occlusion of some of the glands. The essential lesion is a focus of cells which have abundant, faintly-staining, acidophile, non-granular cytoplasm and an oval vesicular nucleus with several nucleoli. The cell shape is usually irregular and frequently the cytoplasm is angular. Very commonly these epithelioid type cells are converted into multi-nucleated cells by an increase in size and a multiplication of nuclei, but such cells still retain their epithelioid appearance. These foci vary considerably in size from a dozen or so cells (Fig. 7) to a definite epithelioid tubercle (Fig. 8) or to a diffuse infiltration of the lamina propria (Figs. 9, 10). The foci frequently appear to be confined into definite follicles by an encircling arrangement of the epithelioid cells. The foci obliterate surrounding glands by peripheral growth. Although not outlined by an inflammatory zone of leucocytic cells, examination of large numbers of sections shows that the cellular content of the surrounding lamina propria is considerably increased in the areas showing lesions.

Giant cells both of the Langhans type and also of the foreign body type are very commonly seen in the lamina propria. They are found associated with the epithelioid infiltrations either centrally or at the periphery, but in addition a number of the Langhans type frequently occur alone or with one or two epithelioid cells around. The giant cells vary considerably in appearance. Sometimes they

are of the classical type, round with abundant acidophil cytoplasm with a considerable number of nuclei arranged in a horse shoe fashion (Fig. 11). Others are irregular in shape with the cytoplasm extending out into long thick projections which penetrate between the surrounding cells (Fig. 12). These branches stain deeply with the basic dyes and owing to the plane at which the section may be cut these deeply staining projections may alone be seen in an epithelioid focus. On occasion and even in isolated giant cells the cytoplasm contains basophilic inclusions which have a laminated appearance (Fig. 13). Sections stained by silver stains show little or no proliferation of reticulin. The total amount of lamina propria involved by these lesions varies considerably from case to case.

The submucous coat shows a considerable variation in the extent of lesions, from a number of scattered tuberculoid foci of epithelioid and giant cells to very extensive involvement especially of the lymphoid follicles with some infiltrations progressing to necrosis (Figs. 14, 15).

There is rarely any involvement of the lymphatic vessels leading to the lymphatic glands but in the latter, lesions are extensive and vary in appearance from case to case. The majority of lesions occur in the cortical zone. The lesions may consist of circumscribed follicles or diffuse areas of epithelioid and giant cells (Figs. 16, 17, 18 . Definite necrosis of the larger lesions commonly occurs (Fig. 19).

Bacteriological examination. In the majority of cases prolonged examination of smears and sections of both bowel and lymphatic glands stained by the Ziehl-Neelsen technique failed to show any acid-fast bacilli but in three cases an occasional acid-fast bacillus was found in smears of the swollen lymphatic gland while in two other cases similar isolated bacilli were found in the epithelioid cell foci of the lamina propria of the bowel. In a number of cases sections of the mesenteric lymphatic glands, stained by Giemsa, show degenerating bacilli in the cellular lesions while similar sections stained by Ziehl-Neelsen's method show the degenerating organisms to be a patchy purple in colour.

Cultural methods show that, up to the present, fourteen cases have given growths of *M. johnei* of the non-chromogenic type. Growth generally takes a considerable time to appear, 6 to 8 weeks or more, and often only one out of the six bottles inoculated gives a positive culture. These fourteen cases include the five already mentioned above where a few acid fast bacilli were demonstrated in smear or section.

Group IV (9 cases)

Post-mortem examination. At autopsy the intestinal lesions may be very similar to those of groups II and III. On occasion, the lymphatic vessels draining the thickened intestine are very obvious in that they are enlarged and show along their course numerous firm nodules (1 to 4 mm. diam.) which are hard and may even be

calcified, the bowel having the appearance of being covered by small tubercles. Cross section of the bowel may also show the presence of similar nodules within the mucosa. One case only showed tremendous thickening of the bowel and associated peritoneum which on section was seen to be due to dense hyaline connective tissue which had replaced most of the normal tissue including the mucous membrane. The associated lymph glands in all cases are grossly swollen and nodular. On section circumscribed lesions of varying size up to 1 cm. diameter may be seen. These lesions may appear caseous and are frequently calcified. This is especially so in the ilio-caecal lymph glands.

Histological examination. The lesions in the mucous membrane are similar to those in Group III in that epithelioid cells and giant cells are the cell types. Many of the lesions in the present series are more definitely circumscribed and tuberculoid than in Group III (Fig. 20) and the lesions situated deep in the mucosa are often encapsulated with a fibrous tissue capsule and show central areas of necrosis, caseation and occasionally calcification (Fig. 21). Sometimes, however, the degenerating tissue does not calcify but becomes laminated or forms bizzare shapes which stain intensely with the basic stains. The giant cells often contain this material which appears to be acting as a foreign body. In addition to these definite nodules diffuse collections of epithelioid cells are also present. Sections stained for reticulin fibres show that the lesions with the exception of one case are not productive since only the fibrous tissue capsule contains reticulin. In one case, much of the mucous membrane, submucosa and peritoneal covering was extensively and diffusely thickened by granulation and fibrous tissue.

The lesions on the peritoneal surface of the bowel and in the afferent lymphatic vessels are very similar to the mucosal lesions. Those in the associated lymph glands are also similar except that caseation, degeneration and calcification is very considerable (Figs. 22, 23) and the lesions are larger so that much of the normal gland is replaced. Encapsulation is very definite (Fig. 24). In addition numerous foci of epithelioid and giant cells without encapsulation are scattered throughout the lymphoid follicles and the sinuses of the medulla are packed with macrophages.

Bacteriological examination. Smears and sections of the bowel stained for *M. johnei* fail to show any typical organisms except in the case showing extensive granulation tissue, but partly acid-fast granules or swollen or broken bacilli may be demonstrated. In sections of lymph glands an occasional acid-fast rod and quite frequently degenerating acid-fast forms can be seen but it is by no means certain that these are *M. johnei.*

Culture on Finlayson's medium was positive in four cases, the organism being of the non pigmented variety.

DISCUSSION

It would appear that Johne's disease in the sheep is not perhaps so simple as the literature would suggest. In the present investigation the disease has been divided into four groups. Groups 1 and 2 can quite definitely be defined in that *M. johnei* was demonstrated in suitably stained smears and sections. On the other hand, in Groups 3 and 4, *M. johnei* could not readily be demonstrated in smears and sections and in many cases could not be demonstrated at all by these methods. In sixteen such cases out of twenty-seven satisfactorily examined cultural examination has, however, shown the presence of *M. johnei*. Of the eleven negative cases, although organisms were not cultured or demonstrated by smear or in section, the histological lesions were considered to be similar to those where *M. johnei* had been demonstrated, while, with the exception of three cases, such sheep were obtained from farms where *M. johnei* had been demonstrated in other sheep. It is considered, therefore, that the evidence suggests that the lesions described in groups 3 and 4 are those of Johne's disease.

If this assumption is correct then the pathology of this disease in the sheep shows considerable variation. The wall of the intestine may be distinctly thickened or it may only be so slightly altered that considerable experience of the normal is required in order to appreciate the abnormality. This supports the findings of McEwen (1939) who stated that in sheep the macroscopic lesions may be insufficient to arouse a suspicion of the disease and of Taylor (1945) who found that in some cases the thickening of the mucous coat may be so slight as to be almost unnoticeable. On the other hand, in the present investigation the macroscopic appearance of the intestines was sometimes very distinctly abnormal with the terminal portion of the small intestine grossly thickened. The occurrence of corded lymphatics as described by McEwen (1939) has been occasionally seen, but the presence of considerable numbers of tubercles and nodules over the serosal surface of the intestine and on the peritoneum does not appear to have been previously recorded. It seems to be generally reported that the mucous membrane of the intestine is not thrown into distinct corrugations as is so often the case in cattle and this is well supported by the present observations. The bowel mucosa may be slightly hyperaemic and have a granular appearance which is by no means obvious and it may show deep cracks when folded over the fingers. As recorded by Wilson (1934) and Taylor(1945) the mucosa may be a deep yellow in colour which if not confused with bile staining makes diagnosis of such cases simple. A review of the literature shows that it is common experience that smears of the mucous membrane of the terminal portion of the small intestine, stained by Ziehl-Neelsen's method, show enormous numbers of *M. johnei* (McEwen, 1939 ; Taylor, 1945 ; Dunkin and Balfour-Jones, 1935 ; Stockman, 1911 ; Twort and Ingram, 1913 ; Howarth, 1932). The present observations suggest that this

is true in only a proportion of cases. In Group I where the mucous membrane was pigmented smears certainly showed enormous numbers of bacilli. In Group 2, where the mucous membrane was non-pigmented, the number of organisms seen in smears varied considerably; in some cases organisms were abundant, in others only a few could be demonstrated over a number of smears.

In Groups 3 and 4, *M. johnei* could not usually be demonstrated in smears from the mucous membrane of the intestine but on occasion odd bacilli could be detected in smears from the enlarged lymph glands. More frequently, however, organisms could not be demonstrated by direct smears of either bowel mucosa or mesenteric lymph glands. It would appear, therefore, that diagnosis of Johne's disease in the sheep by direct smears is not always so easy as previous workers have suggested. Twort and Ingram (1913) in their original transmission experiments in sheep found definite lesions but could demonstrate very few organisms, while McEwen (1939) also appeared to find difficulty in demonstrating bacilli in some cases of obvious clinical infection. It should also be noted that there does not appear to be any great correlation between the degree of macroscopic abnormality in the bowel and the number of organisms which are present on smear. For instance in Group I the macroscopic abnormality tended to be minimal while the number of organisms was very great, while frequently in Groups 3 and 4 the pathological abnormality was comparatively obvious and no organisms were seen. The transverse fissures of the thickened mucous membrane mentioned by McEwen (1939) have frequently been very obvious and yet in these cases, unlike his, *M. johnei* frequently could not be demonstrated.

The mesenteric lymph glands vary considerably in appearance. In Groups 1 and 2 they appeared fairly normal or slightly enlarged whereas in 3 and 4 they were frequently very enlarged and oedematous and not infrequently the cut surface showed areas of necrosis, caseation and even calcification.

The histological appearances of the disease as seen in the present investigation were not nearly so constant as has previously been suggested. For instance Twort and Ingram (1913) stated that the lesions in sheep were essentially similar to those found in cattle and this is supported by more recent workers. M'Fadyean (1918) gave the essential lesion in cattle as a macrophage or epithelioid cell infiltration of the normal tissue which at least in the bovine species never showed any evidence of caseous or calcareous degeneration; moreover, the lesions never showed any of the nodular or encapsulated characters which are seen so commonly in tuberculous lesions. McEwen (1939) stated that the lesions of Johne's disease in sheep whether in the intestine or in the lymph glands were confined to accumulations of one type of cell, namely the macrophage or large epithelioid cell. No cellular reaction other than the accumulation of epithelioid cells occurred, but the epithelioid cells crowded

out and replaced the cells normal to the part and in advanced lesions they caused an almost complete disappearance of the normal cells between the glands from the base of the intestinal epithelium to the muscularis mucosae and also into the submucous coat. In the lymph glands McEwen stated that the greatest number of epithelioid cells were found in the cortical lymph sinuses. The complete absence of necrosis in the lesions in the mucous membrane and submucous coat of the intestine and in the lymph glands was in marked contrast to the necrosis of the cell accumulations in the obstructed lymph vessels. McEwen considered that there was no increase of plasma cells, lymphocytes or eosinophiles which were present only in normal numbers in the mucous membrane until crowded out by the epithelioid cells. The giant cells, which are well recognised in cattle, were rarely found by McEwen in sheep which is in agreement with Lange and Berge (1915) and Gaiger and Davies (1946). The present findings differed considerably from the above. In Group I the histological picture was essentially similar to that described by McEwen except that the involvement of the lymphatic vessels was seen in only two of the eight cases. The lesion in the bowel wall was essentially a proliferation of epithelioid cells containing very large numbers of organisms and with no proliferation of other cell types. In Group 2, although the lesion was essentially similar to Group 1, the production of epithelioid cells was not nearly so prolific and in many cases the impression was gained that the epithelioid cells were tending to a nodular formation and that there was a considerable increase in the number of non-granular leucocytes surrounding these epithelioid foci. The invasion of the submucous coats by macrophages was very limited but there was considerable infiltration with other leucocytes. The afferent lymphatics occasionally showed necrosis and caseation as described by McEwen but more frequently these lesions were absent. The lymphatic glands showed all stages of epithelioid proliferation up to almost complete replacement of all normal structures. In Group 3, considerable numbers of epithelioid tubercles occurred in the lamina propria and these were associated with a very definite proliferation of non-granular leucocytes in the surrounding tissue so that the normal glandular structures were undergoing pressure atrophy. Giant cells both of the Langhans and foreign body type were very commonly seen in association with the epithelioid tubercles and also were found isolated in the lamina propria. The submucous coat was also commonly involved with similar lesions. Necrosis of the intestinal lesions was not uncommon while similar but more advanced lesions were often seen in the cortical lymphoid tissues of the mesenteric lymph glands. In Group 4, the lesions in the intestinal mucosa were essentially similar although larger than in Group 3, but caseation and even calcification was common and fibrous tissue encapsulation of lesions sometimes very definite. Caseous and calcified nodules were on occasion also seen over the

serosal wall of the bowel. The lesions in the mesenteric lymph glands consisted of all stages from epithelioid tubercles to considerable caseation, calcification and fibrosis.

The fact that necrosis, caseation and calcification have been frequently observed in the present investigation would appear to be of considerable interest since previous workers have been very definite that *M. johnei* unlike *M. tuberculosis* will not produce necrosis of the affected tissues. (Twort and Ingram, 1913 ; M'Fadyean, 1918 ; Hallman and Witter, 1933). There has, however, been some evidence apart from the present observations that *M. johnei* may produce lesions of this type. M'Fadyean and Sheather (1916) described lesions of caseation in the mesenteric lymph glands of goats experimentally infected with *M. johnei* but unfortunately tubercle bacilli were also present. Despite this, they believed the caseous lesions were produced by the *M. johnei* and in support of this contention pointed out that in three of four cases of natural Johne's disease in the goat similar lesions were found. Holmbae and Slagsvold (1934) also give evidence that goats infected with *M. johnei* may show caseous nodules similar to those found in tuberculosis although without calcification or encapsulation. More recently Levi (1948) recorded caseous and calcified lesions in the mesenteric glands of experimentally infected goats from which he isolated *M. johnei* but could find no evidence of *M. tuberculosis*. It would appear from the present observation that similar lesions may be present in Johne's disease in sheep.

As already stated a review of the literature on Johne's disease in the sheep indicates that nearly all investigators accept the fact that very large numbers of acid-fast bacilli are found in smears from the intestinal mucosa and that the general pathological change is a simple macrophage infiltration. For instance Twort and Ingram (1913) stated that it was clear that the pathological condition caused by *M. johnei* was strikingly different from that found in tuberculous disease that has existed for any length of time. The absence of caseation was one of the most important differences and indeed cellular degeneration could rarely be recognised. The general diffusion of the lesions and the absence of any fibrous tissue capsule were also distinctive features; but perhaps the most prominent difference between the lesions of Johne's disease and tuberculosis was the large number of causal organisms present in Johne's disease. The same workers, however, in experimental infection of sheep with *M. johnei* taken from the bovine, reported definite lesions but only a few bacilli. McEwen also reported that in one sheep showing symptoms of Johne's disease he had seen typical accumulations of epithelioid cells but no organisms could be demonstrated. He suggested that a resistance or immunity to the johne bacillus may sometimes arise and the bacilli be effectively disposed of while the epithelioid cells remain as witness to the former activity of the infection. This would appear to be the only

reference in the literature to any suggestion of alteration in lesions due to immunity or virulence of organisms. The present observations suggest that variation in lesions in the sheep may be just as considerable as the variations in lesions seen in tuberculosis in various hosts. Diffuse epithelioid cell proliferation, tubercle formation with giant cells, necrosis, caseation, calcification and fibrous tissue encapsulation have been seen, while the number of organisms present varied from enormous numbers through all degrees to none being present.

Rich (1944) stated that two important variables, bacterial virulence and host resistance, determine in large measure the character of the lesions and the course which any infection will pursue.

Variations in bacterial virulence are determined by changes in the constitution of the bacteria and variations in resistance are determined by changes in the constitution of the host. In Johne's disease in the sheep in Scotland there appear to be two types of bacilli, the pigmented and the classical bovine types and there is no evidence that within each group there are any variations in character of different strains. It can be assumed, therefore, that in Johne's disease in the sheep any variation in lesions may be dependent upon either (a) pigmented bacilli or non-pigmented bacilli (b) variations in resistance of individual sheep.

The pathogenicity of any bacterium can be measured by the ability of small numbers of organisms to multiply progressively within the tissues of the host and this is dependent, as already said, upon the virulence of the organism or the resistance of the host. It is evident that in Group 1 *M. johnei* multiplied enormously and although little or no toxic effect on the tissue of the host including the attracted epithelioid cells was caused, there was very considerable mechanical damage to the glandular mucosa of the intestine. The majority of cases in this group were caused by pigmented bacilli and these bacilli both in the present investigation and also in previous reports only gave rise to Group 1 lesions, the histology of which suggests that the host makes little or no attempt to localise the infection in the intestine.

Groups 2, 3 and 4, on the other hand, showed examples of infection where the number of organisms varied from very large numbers to very small numbers which may only be demonstrable by cultural methods. The lesions also varied accordingly from what appeared to be (a) uninhibited multiplication with diffuse epithelioid cell infiltration almost as great as in Group I (b) focal epithelioid cell lesions with comparatively few bacteria and a definite increase in the peripheral round cell leucocytes (c) tubercle formation with or without giant cells and johne bacilli difficult or impossible to demonstrate histologically (d) encapsulation, restricted caseation and calcification and *M. johnei* only shown to be present by cultural methods. The organisms in these three groups were of the

non-pigmented variety and so far as the investigation has gone appeared to be of the classical type.

Since the sheep affected by both the pigmented and non-pigmented varieties do not appear to differ in any way but represent in both cases a cross-section of the sheep population of the area, it can only be assumed that the pigmented variety is more virulent for the sheep than is the classical non-pigmented variety. The fact that the clinical disease with the yellow strain also appears to be on the whole more rapid than that of the classical type supports this view.

It is interesting that in Group 4, *M. johnei* occasionally caused the unusual appearance of necrosis and caseation and yet at the same time the evidence of localisation of lesions was very definite and the number of bacilli very few.

SUMMARY

The pathology of Johne's disease in the sheep as it occurs in Scotland is given. On histological appearances it has been divided into four groups.

Group 1 is characterised by masses of epithelioid cells. The pigmented variety of *M. johnei* is most commonly present in very large numbers.

Group 2 is characterised by a varying extent and distribution of epithelioid cell infiltration along with considerable leucocytic cell increase. The number of organisms seen in smears are usually not nearly so numerous as in Group 1. In sections, *M. johnei* is present in the larger areas of cellular infiltration but not in the smaller discrete foci.

Group 3 is characterised by foci of epithelioid and multinucleate cells, along with giant cells of the Langhans type. The foci may vary from a dozen or so cells to a diffuse infiltration of the lamina propria. In the majority of cases *M. johnei* cannot be demonstrated except by cultural examination.

Group 4 is characterised by lesions similar to those described in Group 3 but encapsulation, necrosis, caseation and calcification are present. *M. johnei* can only be demonstrated in a limited number of cases even by cultural methods.

The evidence suggests that the pigmented variety of *M. johnei* is more virulent for the sheep than is the classical strain.

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Fig. 1

Fig. 2

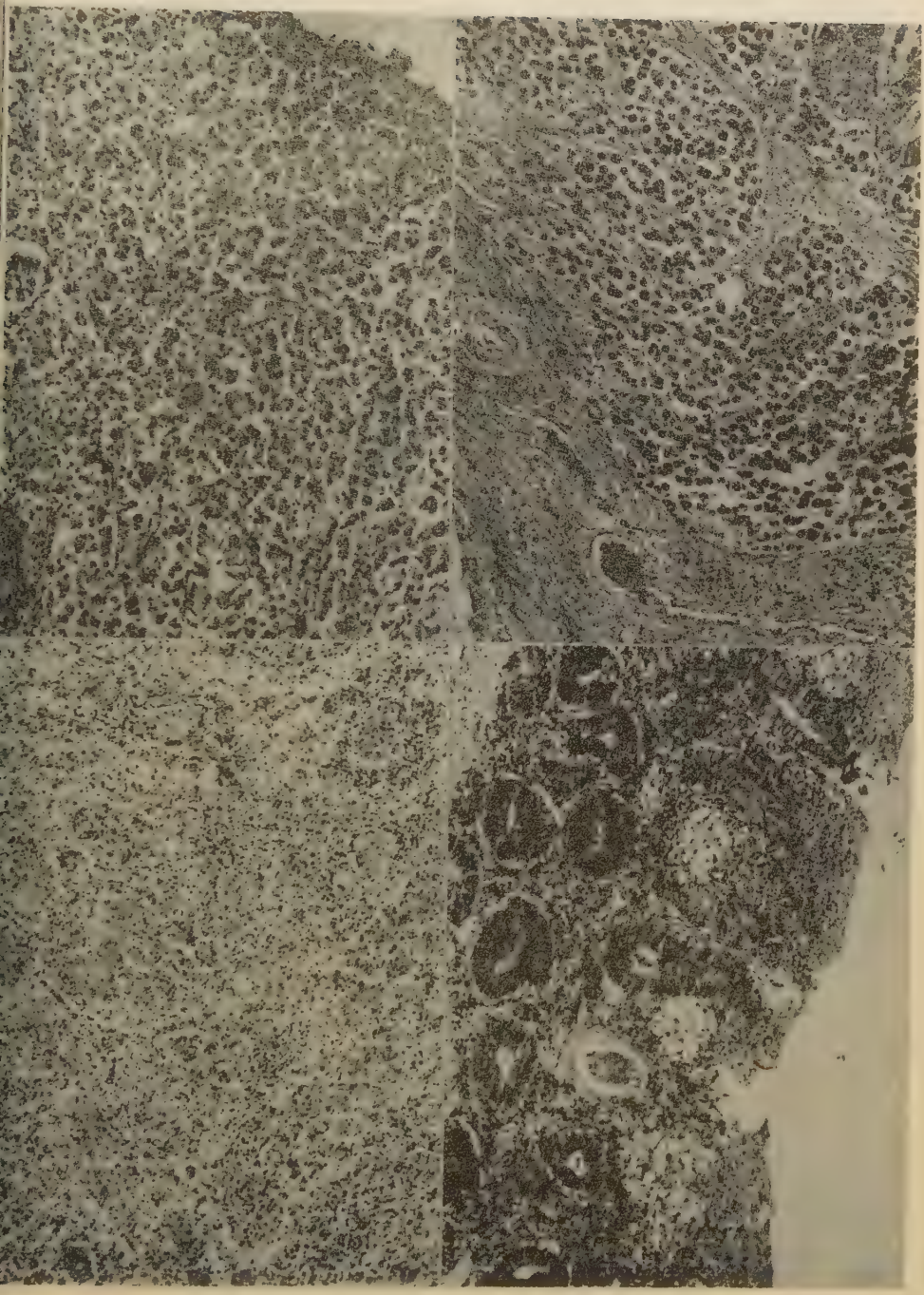

Fig. 3

Fig. 4

Fig. 5

Fig. 6

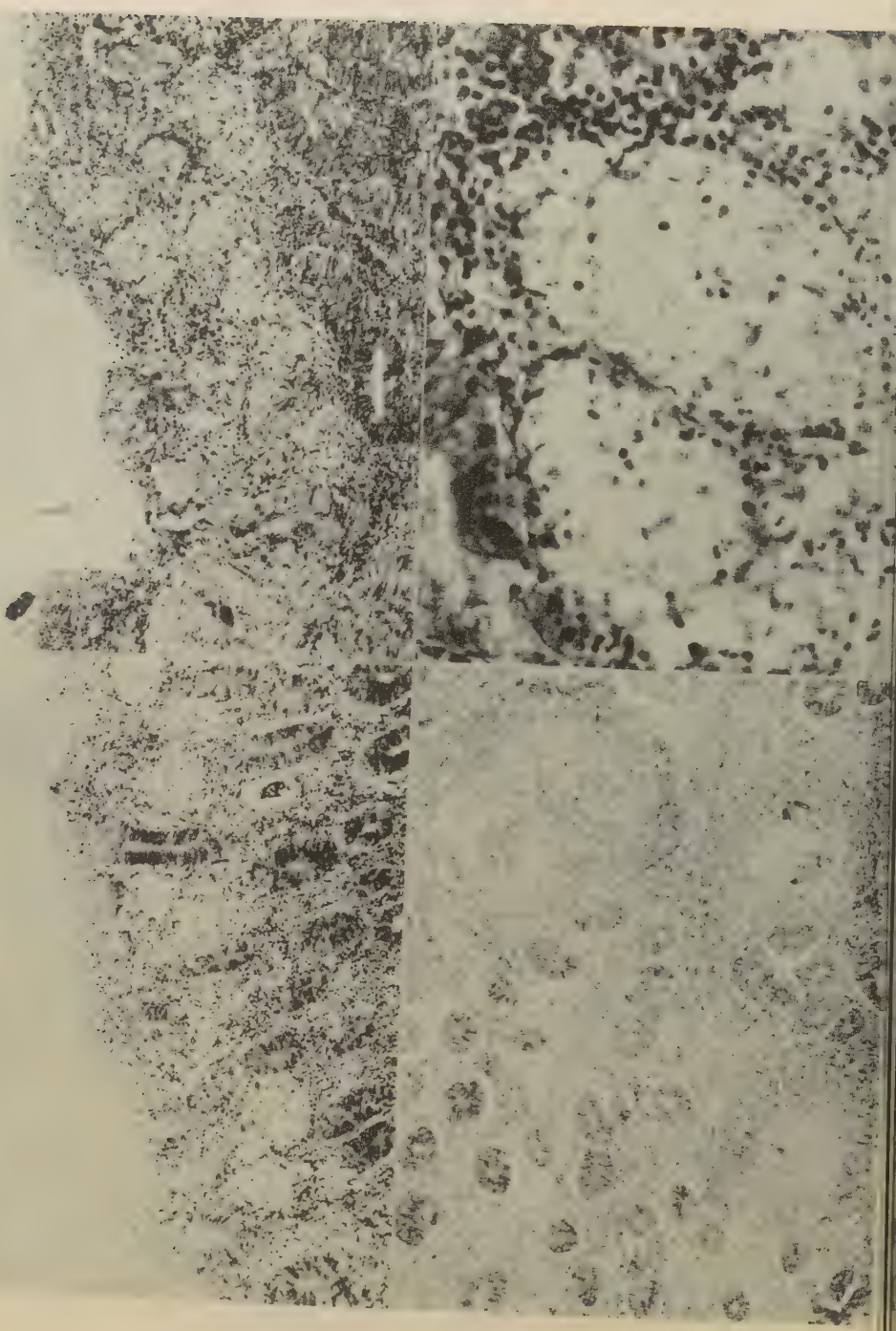

Fig. 7

Fig. 8

Fig. 9

Fig. 10

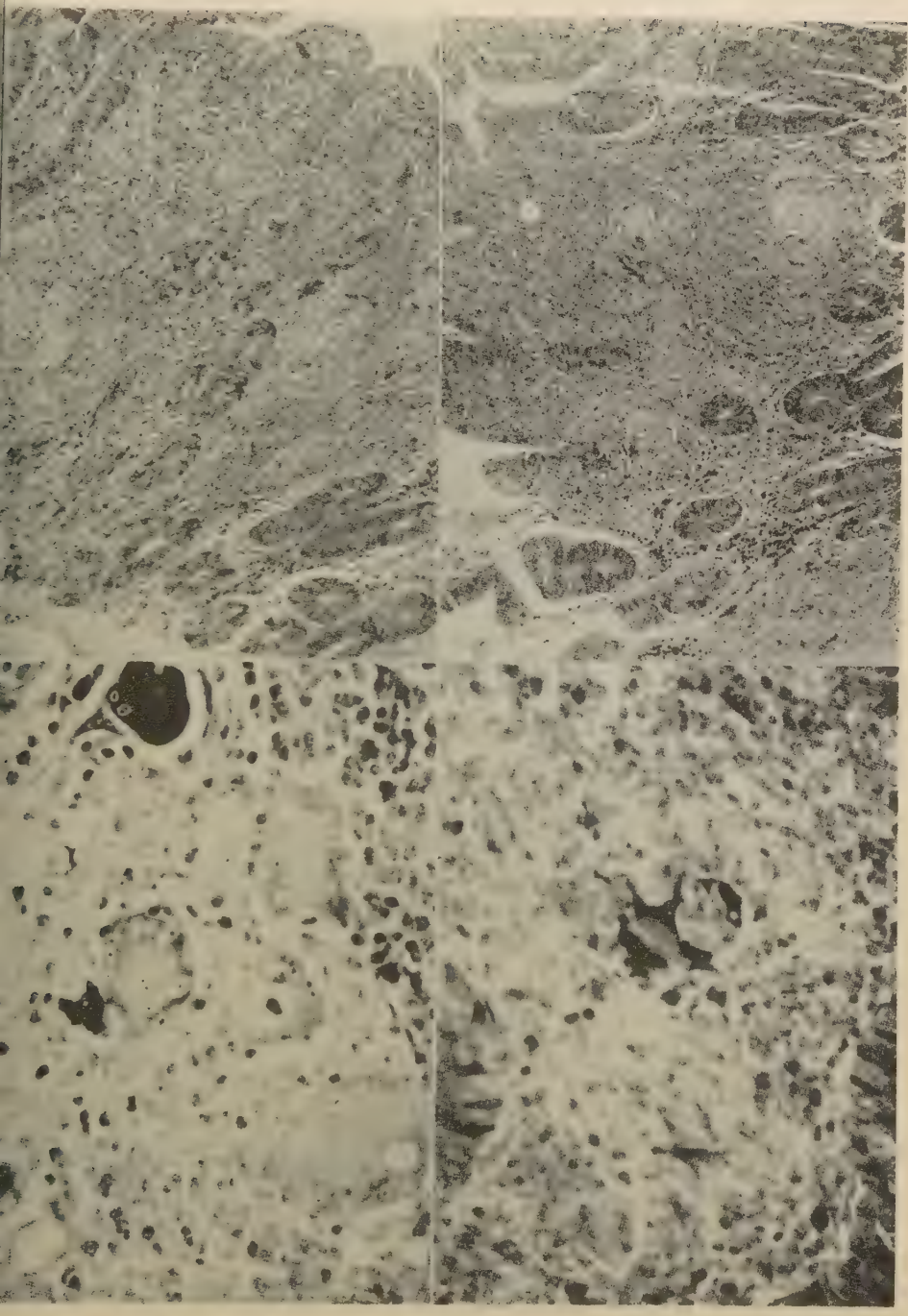

Fig. 11

Fig. 12

Fig. 13

Fig. 14

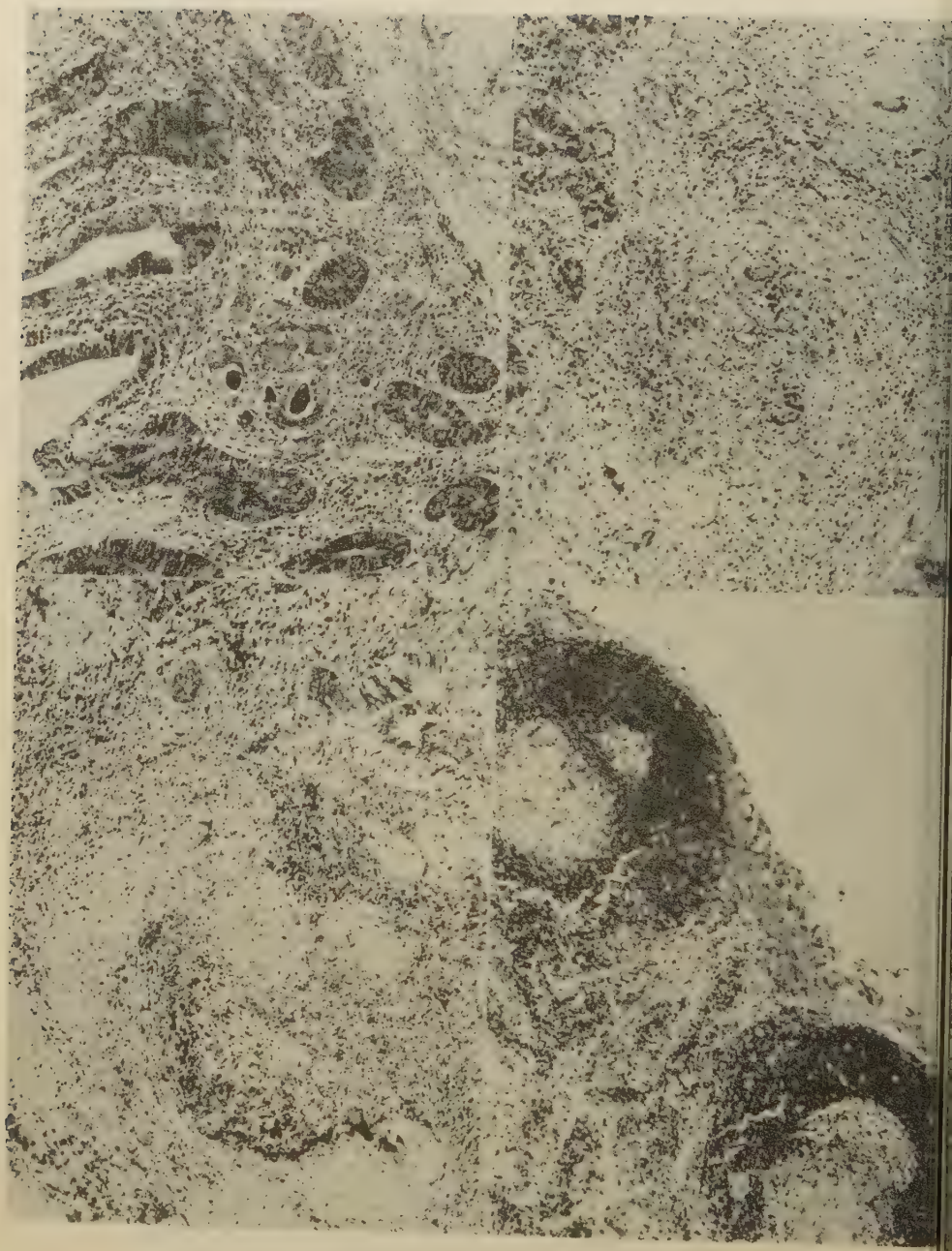

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Fig. 19

Fig. 20.

Fig. 21

Fig. 22

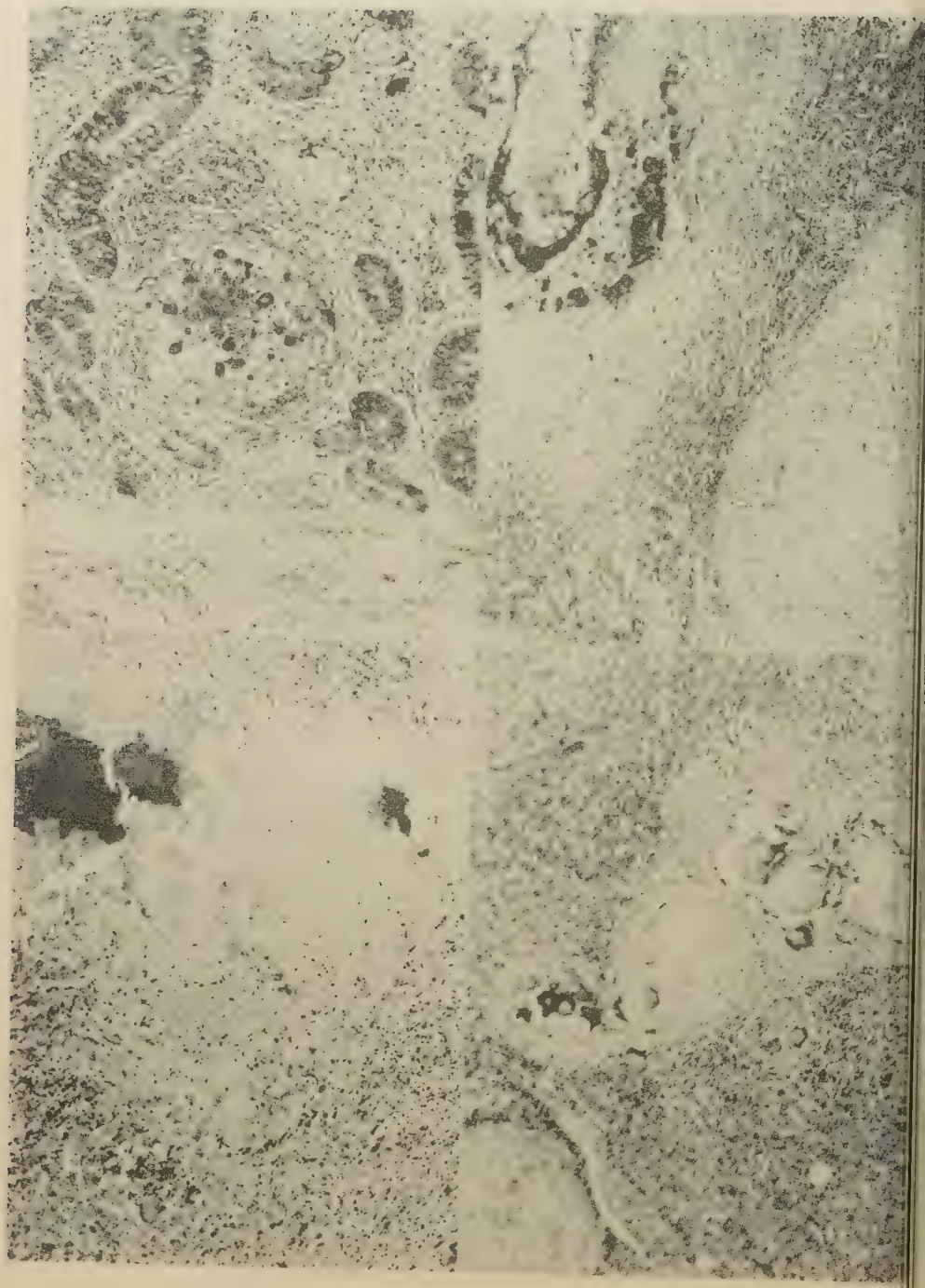

Fig. 23

Fig. 24

To face p.

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LEGENDS TO FIGURES.

Fig. 1. Structures of the lamina propria replaced by epithelioid cell masses.
Group 1 H & E.
X 100

Fig. 2. Epithelioid cells invading through the mucularis mucosae to the submucosa.
Group 1 H & E
X 100

Fig. 3. Epithelioid cell and leucocytic infiltration of mucous membrane.
Group 2 H & E
X 100

Fig. 4. Foci of epithelioid cells, each focus consisting of a few cells.
Group 2 H & E
X 100

Figs. 5 & 6. Multiple nodules of epithelioid cells.
Group 2 H & E
X 100
X 400

Fig. 7. Foci of epithelioid and multinucleated cells.
Group 3 H & E
X 100

Fig. 8. Tuberculoid lesions.
Group 3 H & E
X 100

Fig. 9 & 10. Diffuse infiltration of the lamina propria with epithelioid and giant cells.
Group 3 H & E
X 100

Fig. 11. Giant cells of the Langhans type.
Group 3 H & E
X 400

Fig. 12. Giant cell of the "foreign body" type.
Group 3 H & E
X 400

Fig. 13. Giant cells containing basophilic inclusions.
Group 3 H & E
X 100

Fig. 14 & 15. Submucous coat infiltrated with epithelioid and giant cells.
Group 3 H & E
X 100

Fig. 16, 17 & 18. Varying degrees of cellular infiltration of mesenteric lymph glands.
Group 3 H & E
X 100

Fig. 19. Cellular infiltration progressing to necrosis.
Group 3 H & E
X 100

Fig. 20 & 21. Encapsulated tuberculoid lesions of lamina propria.
Group 4 H & E
X 100

Fig. 22 & 23. Caseation and calcification in mesenteric lymph glands.
Group 4 H & E
X 100

Fig. 24. Encapsulation of a mesenteric lymph gland lesion.
Group 4 H & E
X 100

ROUND HEART DISEASE IN THE FOWL

By

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INTRODUCTION

This disease, of relatively recent origin, characterised by the sudden death of what appear to be normal birds, often under a year old, has attracted the attention of workers in several countries but little real progress has been made towards determining its aetiology. It is known by various names suggested either by the unusual appearance of the affected heart or the alarming nature of the symptoms: in Great Britain, Round Heart Disease, in New Zealand, Enzootic Syncope of Fowls or Toxic Heart Degeneration (Fischel, 1946), in Germany, "Eierherzen" or Egg Heart (Eber and Pallaske-Eber, 1934) and in Denmark, Yellow Heart Degeneration (Adsersen, 1948). Other reports are : from Germany (Matzke, 1942), Northern Ireland (Luke, 1947), England (Blaxland and Markson, 1947) and Scotland (Wilson, 1947, 1948).

The earliest outbreak in Scotland (Wilson, 1948) occurred on a pedigree poultry farm in Perthshire in 1942. No cases occurred in the following year but there was a recurrence in 1944. On both occasions White Leghorn pullets under a year old were affected but Rhode Island Red pullets in close contact remained healthy. In 1943 the disease was diagnosed in the inbred flock of Brown Leghorns maintained for the purpose of genetical research at the Institute of Animal Genetics. Cases occurred every year until 1948. From the pedigree records which extend back for many years it appeared that the disease was most prominent in a line carrying a high proportion of blood from a Danish cock which had been imported in 1931. The possibility of a hereditary basis for the disease was discussed.

DESCRIPTION

Present Position

It was hoped to continue the investigation into the genetical aspect of the disease but from 1948 no cases occurred in the flock at the Institute of Animal Genetics until the spring of this year when two birds died from it within a week of each other; since then no further cases have been seen. Within the last few years Round Heart disease has become much more common in Scotland and the North of England. From 1945 to 1949 there were 10 outbreaks, in 1950 there were eight and in 1951, 11. In 1952 there was a spectacular rise in incidence with 19 recorded outbreaks and during the first six months of this year a further rise to 29. The investigation of these outbreaks has shed fresh light on some interesting aspects of the

disease and provided a large amount of material for histopathological examination. The symptoms in some of the more recent cases differed from those previously described but generally speaking there is little discrepancy between the earlier and later outbreaks.

The disease was confined to Leghorns, Rhode Island Reds and their respective crosses and was not seen in Light Sussex, White Wyandotte, Buff Rocks and other popular breeds even when they were kept in close contact with affected fowls. In many outbreaks the selectivity of the disease was the most striking feature, e.g., of several strains of White Leghorns kept under apparently identical conditions, only one was affected. On other farms Leghorns were affected and Rhode Island Reds remained healthy and vice versa. A very striking case occurred in a deep litter house in two large groups of birds obtained from different sources and separated by a wire partition only. The attendant regularly crossed from one compartment to the other and the food and water were from a common supply. One group showed a high death rate and in the other not a single case occurred. In another outbreak there was a 25 per cent. death rate in a single pen of Brown Leghorns kept intensively but no cases occurred elsewhere on the farm. On the other hand there were instances in which birds of unrelated strains of Leghorns or Rhode Island Reds or both on the same farm succumbed. In one very large outbreak the disease had every appearance of an infection and spread from one group to another in adjacent deep litter houses. On another farm where pullets of four different Leghorn crosses were kept together the disease appeared simultaneously in all breeds and attacked them indiscriminately.

The disease appeared to occur under all the usual systems of poultry husbandry except that none of our outbreaks involved birds in laying batteries. Many of the recent outbreaks were in birds kept on deep litter. There may be no real significance in this, however, since the greater incidence coincided with a period of increased popularity of this particular system. A survey of the circumstances of the various outbreaks investigated yielded strong support for a hereditary basis in some; in others, where different strains and even breeds were attacked indiscriminately, it was apparent that some other factor was involved.

Seasonal Incidence

In an earlier paper attention was drawn to the striking incidence of the disease which was prevalent from January to April with a peak in February (Wilson, 1948). In Denmark and New Zealand most outbreaks also occurred during this period but in Northern Ireland from June to November was the critical time. It is interesting to note (Table I) that the later outbreaks that we have observed show the same seasonal incidence as the earlier ones. Until 1953, February was usually the peak period but this year a very much higher incidence occurred in January. There is a relative absence

TABLE I

SEASONAL INCIDENCE OF ROUND HEART DISEASE

	Jan.	*Feb.*	*Mar.*	*Apr.*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1945											1	
1947			1	1		2						
1949		2	1	1	1							
1950	3	1	2			1						1
1951	1	3	2	3				1		1		
1952	1	6	4	3					2	1	2	
1953	16	5	4	2	2							
	21	17	14	10	3	3	–	1	2	2	3	1

of the disease during the summer months with complete freedom during July.

Symptoms

The symptoms of the early outbreaks were given previously (Wilson, 1948) and may be briefly summarized. Affected birds were generally in good condition; death was almost always sudden and often occurred when birds were excited, as at feeding time. In the rare cases in which signs of illness were observed they occurred from a few hours to a day or so before death. Affected birds were rarely in lay; the period between the laying of the last egg and death varied from 1 to 195 days; in about half the cases it was between 4 and 30 days with an average of 15 days. The disease was most common in pullets but cases were observed in older birds. Males were rarely affected; it is unlikely that the relatively small number of males normally retained provides an adequate explanation of this for on some farms quite large numbers of cockerels of a susceptible age were kept.

Such a history is still typical of most outbreaks but in some the severity varies greatly. At first deaths may be sudden and without prior symptoms. After a week or even more a few ailing birds may be detected. Symptoms at first may be very slight and easily overlooked by an inexperienced observer but within a few days they become obvious. The affected bird is less active and the tips of the comb and the borders of the wattles may be slightly cyanosed. This becomes more marked and the comb and wattles lose their sheen, the epidermis becoming dry and later, scaly. There is inappetance followed by loss of condition and finally the comb and wattles become shrunken. Birds are reluctant to move and remain in corners. Sometimes on entering a pen to catch an affected bird of this type there is a sudden flapping of wings and an apparently healthy bird drops dead from typical Round Heart disease. In other outbreaks no sudden deaths occur but a proportion of the flock shows

the symptoms just described. This is the least common type of the disease. It appears that recovery may take place if the disease is not too advanced. The survivors of a serious outbreak which had caused many deaths in a particular group of fowls were obtained and kept at the laboratory. Several were obviously ill and a few died within a week but the remainder showed an almost immediate and startling improvement in health. As the owner was unwilling to take any of the birds back it was not possible to control this observation but it is certain that many more would have died if the birds had not been moved.

There is no ready method of making a definite diagnosis in the living bird. X-ray photography and screening have been attempted but both methods are unsatisfactory.

Post-Mortem Findings

Except in birds which have been ailing before death, bodily condition is always good and food is almost invariably present in the crop. The most striking changes are seen in the heart but these vary considerably with the severity and duration of the disease. In advanced cases the organ is greatly enlarged and may be twice the normal weight. The typical conical appearance is completely lost. The organ becomes egg-shaped or barrel-shaped and the apex is flattened or may have an indentation or "dimple" of varying depth in the centre. The muscle fibres around the dimple assume a spiral or whorl-like appearance. The dilated heart has a characteristic pinkish-yellow par-boiled appearance and the ventricles are flabby in consistency. The disease is more marked in the left ventricle but the right ventricle is also involved. The cut surface may present a patchy appearance, the affected areas being lighter in colour than the normal muscle but in advanced cases the entire wall of the ventricle may be affected.

In less advanced cases the dilation of the heart is not so marked, the discoloration of the ventricle walls is patchy and there is no dimpling of the apex. In still earlier cases where death may supervene as a result of some sudden or severe exertion, the degree of dilatation may be small and could be overlooked by an inexperienced observer. The various alterations in the shape of the heart most commonly observed are shown in the accompanying photographs.

The coronary veins and their branches are markedly congested and stand out prominently on the surface of the heart. Petechial haemorrhages are sometimes seen but are not common. The heart valves invariably appear normal and there is no evidence of endocarditis. Hydrops pericardii is sometimes striking. The pericardial sac may be distended enormously with clear sero-gelatinous straw coloured effusion, sometimes containing large strands of fibrin. In addition to these changes in the heart the large veins of the general circulation are distended with blood and there is always an intense terminal passive congestion of the internal organs. The greatly

increased amount of blood in the liver causes the capsule to be tightly stretched and the organ presents a very characteristic glistening almost metallic appearance. There may be a low grade ascites and strands of fibrin may be found on the liver surface. The lungs are sometimes congested and oedematous but on occasion appear almost normal. The kidneys are always very congested to such an extent as to give the organs a blackish appearance. The ovary is also acutely congested. Fischel (1946) found that the skeletal muscles were pale and slightly moist but this was not evident in our case.

Histological Examination

The hearts and other organs from 70 cases of Round Heart disease of varying severity were selected for detailed histological examination. For routine purposes tissues were fixed in 10 per cent. formalin and embedded in paraffin. Sections were stained by haematoxylin and eosin. Frozen sections were also cut and other specialised staining techniques were employed as will be discussed in the text.

All sections of affected hearts showed the typical changes which are considered to be characteristic of Round Heart disease. The distribution of the lesions depended on the severity of the disease. In advanced cases the whole of the myocardium was almost equally affected. In early cases and in intermediate cases the lesions were confined to areas in the wall of the ventricles. The subendothelial portions of the myocardium showed the least advanced lesions; the central part of the heart wall was more obviously altered. The patchy distribution of the degenerate areas is reminiscent of the lesions found in the hearts of pigs dead from cardiac syncope or of calves suffering from avitaminosis E which are attributable to Zenker's degeneration. Fischel (1946) failed to find evidence of this degeneration in cases of Round Heart disease and hyalin was not demonstrated in any of the series now reviewed by haematoxylin and eosin, Russel's method for hyalin (Carleton and Leach, 1938) or the technique with Phloxin B as described by Lillie (1948). Morris (1952) stated that some hearts "appeared uniformly inflamed" and Blaxland and Markson (1947) described an acute diffuse myocarditis. In our view inflammatory changes in the heart are not characteristic of the disease and no evidence of the typical changes associated with inflammation of the heart was obtained. The pathological changes both macroscopical and microscopical are those of a degenerative process. Our observations regarding cellular infiltrations are in agreement with those of Adsersen (1948) who is of the opinion that they occur only very occasionally.

In most of the affected fibres there is loss of striation but this is not considered to be primary as early nuclear damage may be seen while the remainder of the fibre is still normal. Alterations in

nuclei, therefore, would appear to precede cytoplasmic changes As the nuclear changes progress the striations disappear and further retrogressive changes become manifest. Although cloudy swelling has been described regularly by other workers, true cloudy swelling was not noted in the series of cases investigated. The nuclear changes and loss of striations are followed by more or less severe fatty change of a degenerative nature. This may be demonstrated by the usual fat stains. Fat droplets are small at first but appear later to become confluent and may then be recognised by ordinary staining with haematoxylin and eosin. It is difficult to differentiate them, however, from other degenerative changes by this method. Fatty change does not appear to represent the end of the degenerative changes and we suggest that it occurs fairly early in the disease. The smaller fat globules are present in the fibres showing early degenerative changes whereas fat cannot be demonstrated in the swollen fibres to anything like the same extent. This suggests that it is replaced by later retrogressive processes. The intercellular fatty infiltration described by Marzke (1942) and Blaxland and Markson (1947) was not evident in our cases.

The heart muscle fibres become greatly distended and may reach 4 to 5 times their normal diameter. Their affinity for eosin is reduced. The whole of the myoplasm may be replaced by a fine network, forming vacuoles of varying size which may occasionally coalesce to form large empty spaces within the fibre. This reticular appearance suggests that an intracellular oedema succeeds the fatty change. Although Zenker's degeneration does not occur, isolated patches of homogeneous material are sometimes seen. These stain more deeply with eosin but do not give the staining reactions of hyalin. The myofibrils are lost in damaged fibres. This is well demonstrated in the transverse section of fibres which in many cases show only a faint reticular arrangement in the centre with very little normal cytoplasm. Sometimes the reticulation may have disappeared completely from the centre of the fibre leaving it structureless, the cytoplasm being concentrated round the periphery producing a "ring-like" appearance. The Purkinje fibres were found to be normal.

Hyperchromasia (margination of the chromatin round the nuclear membrane) was a frequent and characteristic finding in the myocardial nuclei, but the nuclear degenerations in the form of pyknosis and karyolysis described by Fischel (1946) were not seen. The normal nucleus of the myocardial cell is elongated and the nucleolus is small and not very distinct and it is difficult to differentiate it from other chromatin material. In Round Heart disease the nuclei become oval but still retain their normal staining properties. As they become more rounded they stain less intensely. As the process continues they become very dilated and almost spherical and the chromatin is concentrated round the periphery of the nuclear membrane. Finally, the edges of the nuclei take on a

crenated appearance; this form is seen in the severely damaged and greatly thickened fibres.

One, or less commonly, two or more, round or oval inclusions are seen in the nuclei of affected fibres from the early stages of nuclear degeneration onwards. The size and position of the inclusion vary. Sometimes it occupies most of the nuclear space and becomes surrounded by a "halo" when the chromatin marginates round the nuclear membrane. The surface of the larger inclusions sometimes presents a somewhat pitted appearance. Their affinity for basic stains is similar to that of the remainder of the nucleus and they cannot be differentiated by Mann's, Lentz and Mann's and Gerlach's staining methods. The method of Feulgen and Rosenbeck (1914), however, provides a basis for differentiation; the inclusions are Feulgen negative whereas the chromatin, whether in the granular form in early, or marginated in advanced, cases, and the nucleus are always Feulgen positive. For the purpose of histological diagnosis it is unnecessary to employ any stain other than the ordinary haematoxylin and eosin. The nature of the inclusions is not clear. They may represent degenerated nucleoprotein and be associated with the process of hyperchromasia. It is unlikely that they are of viral origin; further investigations are proceeding.

The hearts of 60 birds selected at random from routine post-mortem specimens and including a proportion which showed cardiac abnormalities other than Round Heart disease were examined histologically but none showed any evidence of the lesions we consider characteristic of Round Heart disease.

The livers, kidneys and spleens of birds dead from Round Heart disease were also examined histologically but apart from a severe passive congestion no pathological changes which could be connected with the heart condition were found. Congestion was so acute that there was not enough time for pressure atrophy or diapedesis to take place. Because of a possible connection with an endocrine upset the pituitary and adrenal glands from a few selected cases were also examined but no abnormalities were found. In view of the similarity between Round Heart disease of poultry and so-called "Herztod" or cardiac syncope in pigs which Doberstein and Mathias (1943) associated with a primary "Thyrotoxicosis", the thyroid glands of a number of birds which had died from Round Heart disease were examined but all appeared to be normal and functional.

DISCUSSION

There is no doubt that the heart lesions are primary but to provide further confirmation, numbers of apparently healthy birds were obtained for post-mortem examination from farms where the disease existed. In a number there were definite lesions of Round Heart disease. Some were in the earliest stages; in others the

disease was quite advanced. There was no evidence of venous congestion of the internal organs.

The disease is primarily a cardiac one of other than valvular origin. It would appear that the noxa, whatever its nature may be, acts on the myocardium causing a primary degeneration followed by dilatation. As a result of degeneration there is impaired output and an increased intracardial pressure which the heart wall, in its diseased condition, cannot withstand. A purely mechanical dilatation is thus brought about by the inability of the damaged heart muscle to compensate. The formation of the "dimple" already described is an extension of this process. In the fowl the arrangement of the chordae tendinae and the musculi papillares differs from that of mammals. The musculi papillares are represented by longitudinal ridges extending the entire length of the ventricle and although they suffer some damage they are not so severely affected by the retrogressive changes as the rest of the myocardium. When the remainder of the left ventricle succumbs to the dilatation as a result of this selective weakening, the pillars and the apex resist leaving the characteristic depression.

The similarity between Round Heart disease and "Herztod" or cardiac syncope of pigs has already been pointed out. (For a complete and up-to-date review of the latter disease see Glasser, 1953). The clinical picture in these two diseases is not unlike, in that both pigs and fowls die very suddenly particularly after some exertion. The macroscopical changes seen at post-mortem examination are also similar. There are irregular pale areas affecting mainly the left ventricle and hydropericardium and ascites are also reported. Histologically, however, the two conditions are quite distinct. Many theories have been advanced concerning a possible cause of syncope in pigs but little proof has so far been produced in support and most can be discounted as possible causes of Round Heart disease.

The possibility of Round Heart disease having a nutritional aetiology cannot be overlooked. The well marked seasonal incidence with no cases in mid-summer, relatively few in autumn and early winter and a peak period in January and February, is interesting and might suggest some association with an essential food factor readily available in spring and summer but not present in sufficient amounts during winter to meet the varied needs of all members of a flock. A straightforward deficiency in the diet is probably unlikely but some interference in absorption or utilisation or the destruction of some essential factor during the storage of foodstuffs are all possibilities. The greater incidence of the disease recently noted in birds kept on the deep litter system has already been pointed out. This might support the theory of some dietetic deficiency but the increased number of cases may be relative rather than real. The history that the disease disappears when birds are allowed free range is also inconclusive since sudden cessation apart from such a change is one of the characteristics of the disease. As we were unable to find

an owner who was willing to put half an affected group outside and allow the rest to remain on deep litter, we were unable to check this point.

The association of vitamin E and cardiac function in a number of animals is now well established. There is, however, no record of a vitamin E deficiency causing cardiac damage in fowls. Most work has been done with young chicks in the investigation of encephalomalacia and exudative diathesis. Seifried and Heidegger (1936) investigated a muscular dystrophy of young ducks now known to be an avitaminosis E (Jungherr, 1936) and described typical Zenker's degeneration in the skeletal and gizzard muscles; there is no record of any alteration in heart muscle. Experiments are at present in progress to investigate any possible connection between vitamin E and Round Heart disease.

Seifried (1943) described lesions in the hearts of calves, sheep, goats and pigs which he attributed to a vitamin C deficiency characterised by both Zenker's and fatty degeneration of the heart affecting mainly the left ventricle. There are also records of cardiac lesions associated with a Thiamin deficiency. McCarrion (1928) found dilatation of the right heart in pigeons while Sherwood and Cough (1948) described somewhat similar lesions accompanied by oedema in the tissues. Follis (1948) supported the view that lack of vitamin B_1 causes an acute heart failure and recently Bolle (1950) suggested that this might be the cause of cardiac syncope in pigs as a result of the destruction of the vitamin through prolonged steaming of the food. So far it has not been possible to connect any of these deficiencies with Round Heart disease but experimental work along these lines has been planned.

Following the demonstration of intranuclear inclusions which do not appear to have been described by other authors, the possibility of a virus causation was reconsidered. The history of some of the field outbreaks is not inconsistent with this. The sudden onset, followed later by much less severe symptoms and the method of spread throughout the flock in some cases is suggestive. On the other hand the behaviour of the disease in other flocks and the failure of transmission experiments recorded by Fischel (1946), Blaxland and Markson (1947), Luke (1947) and Wilson (1948) would appear to discount the virus theory.

Pearce (1950), however, showed that several viruses which did not damage the heart of rabbits when injected intra-testicularly, produced myocarditis and some endocarditis when the oxygen supply was reduced simultaneously with virus introduction. The lesions in the heart muscle consisted of a marked infiltration with inflammatory cells. Intranuclear inclusions were seen and there was some haemorrhage and oedema with necrosis of the muscle fibres. The condition is obviously quite different from Round Heart disease, but the method of its reproduction is of interest in considering possible aetiology.

The history of some outbreaks provides support for the theory postulated after the investigation into the original outbreaks that the disease has a hereditary basis and that some strains of birds are more liable. It has already been noted that the disease was particularly prevalent in the offspring of an imported Danish cock. In one line sired by a grandson it was present but it could not be traced to another line sired by a great-grandson. There was no record of Round Heart disease on the farm in Denmark from which the bird came but cases had occurred in the progeny of cockerels obtained from the farm. On one farm the disease occurred in three separate years in related stock and on two other farms it was recorded twice. It has also been found in the offspring of two cockerels purchased from a farm where the disease was known to exist. These happenings might be considered to provide additional support for the idea that the genetic constitution of the stock plays some part in determining the susceptibility or resistance to Round Heart disease.

As a result of the review of all the outbreaks it has not been possible to determine a common factor nor to reconcile the random and indiscriminate appearance of the disease in some flocks and its restricted occurrence in others. The problem of aetiology is being approached along the main lines—nutritional and genetical—the former by attempting to produce and study certain dietetic deficiencies, chiefly Thiamin and Vitamin E and the latter by inbreeding experiments using a known susceptible strain of fowls.

SUMMARY

Within recent years Round Heart disease has become much commoner in Scotland and the North of England. In 1952 there were 19 outbreaks (almost twice the number recorded in 1951) and in the first six months of this year there were 29. There is a well marked seasonal incidence. Most cases occur in late winter and spring with a peak period in January or February.

The history of outbreaks is conflicting. In some there are indications of genetic selectivity; in others birds are attacked indiscriminately. Detailed accounts of the symptoms, pathology and histopathology of the disease are given and possible aetiological factors are discussed.

ACKNOWLEDGMENTS

We wish to acknowledge with gratitude our indebtedness to Dr. Stableforth for his help at all times. Thanks are also due to our colleagues in the various Animal Health Divisions in Scotland and the North of England for their interest and help in field investigations and to Mr. R. C. Hood, A.I.B.P., of the Royal (Dick) School of Veterinary Studies for the photographs.

Fig. 1

Fig. 2

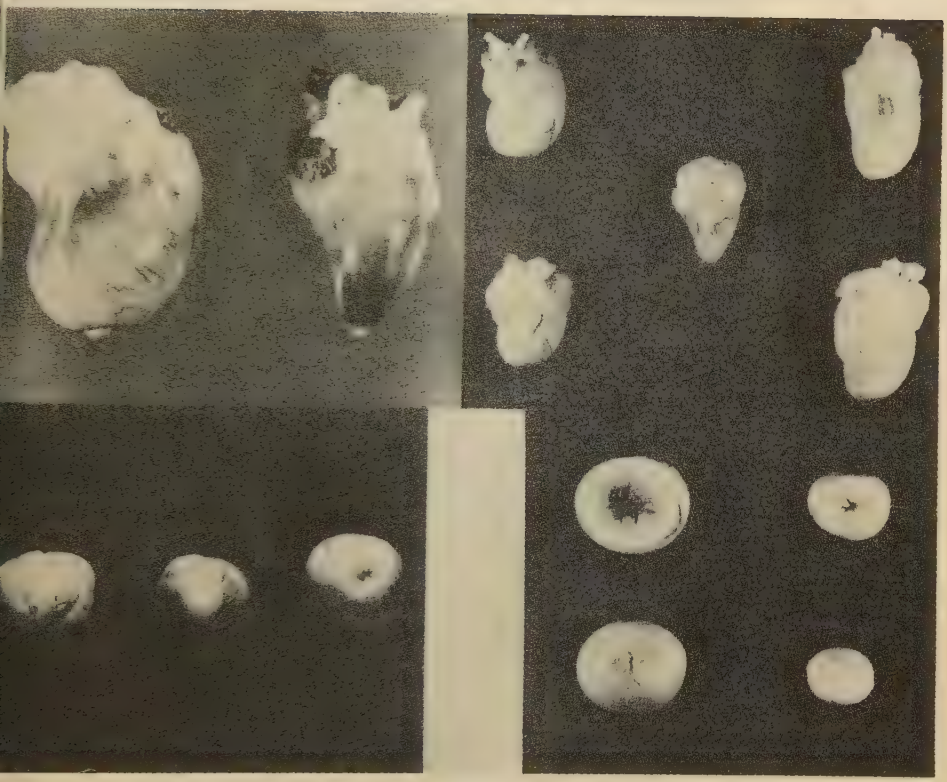


Fig. 3

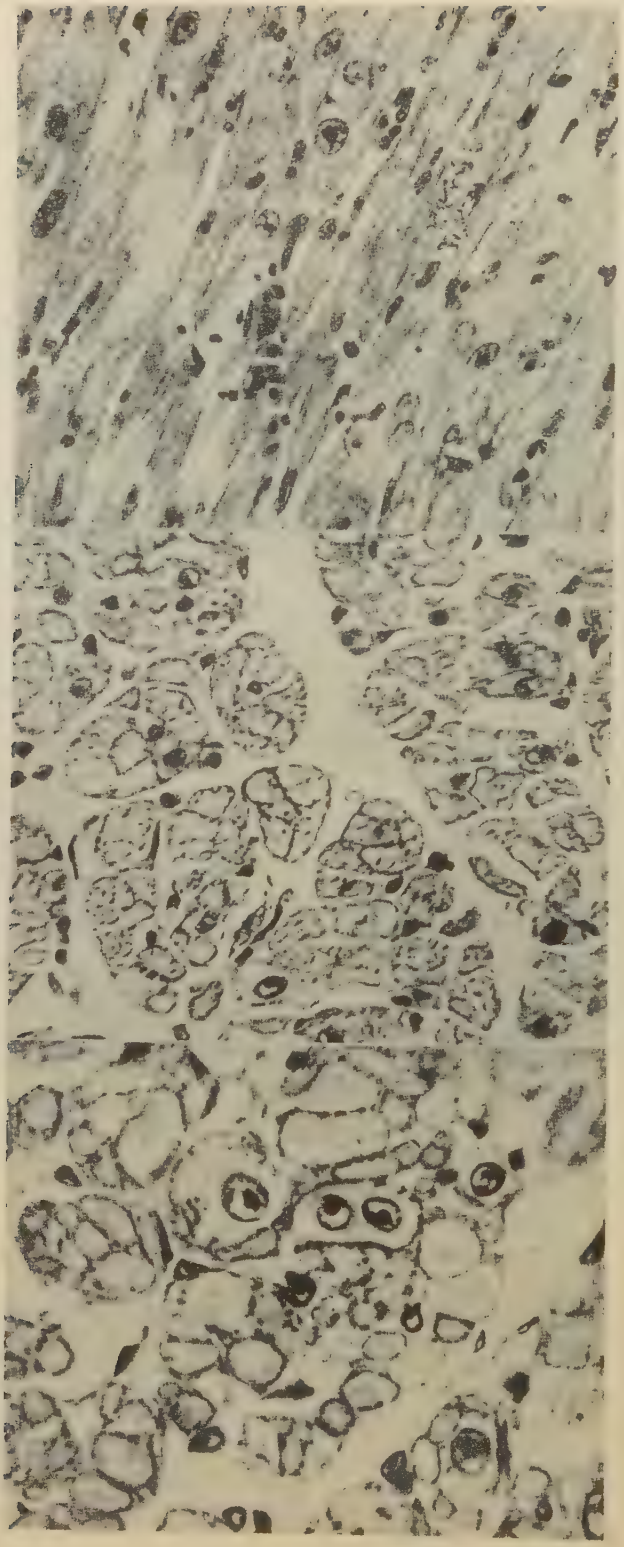
Fig. 4

To face page 50

Fig. 5

Fig. 6

Fig. 7



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LEGENDS TO ILLUSTRATIONS

Fig. 1. Advanced Round Heart Disease.
Note barrel shaped heart with indented apex. The other heart is normal.

Fig. 2. Variation and shape of affected hearts in advanced Round Heart Disease. The heart in the centre is normal.

Fig. 3. Apex of affected heart with normal in centre for comparison. The specimens are from top left and bottom left hearts shown in Fig. 2.

Fig. 4. Sections of affected heart. Left is diseased. Note abnormal apex and dilatation of left ventricle.

Fig. 5. Early Round Heart Disease—fibres cut longitudinally. Striations are still present in some. Others are degenerate with granulation and vacuolation. The nuclei are rounding and undergoing margination and intranuclear inclusions may be seen.
H & E. X 1200.

Fig. 6. Advanced Round Heart Disease—fibres cut transversely. "Ring formation" with concentration of cytoplasm round the periphery of the fibres. Note early crenation of hyperchromatic nuclear wall.
H & E. X 1200.

Fig. 7. Advanced Round Heart Disease—fibres cut transversely. Loss of myofibrils. Large intranuclear inclusions surrounded by "halo" and marginated chromatin.
H & E. X 2000.

A CASE OF SUSPECTED JOHNE'S DISEASE IN A LLAMA (*L. GLAMA*)

By

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INTRODUCTION

Johne's disease is a condition affecting cattle and sheep in Europe, North America and Asia. It has also been reported in goats by M'Fadyean and Sheather (1916), and by other workers. An experimental study of the disease in these animals was made by Levi (1948).

There are few recorded instances of the occurrence of the disease in other species. M'Fadyean (1907) reported a case in a park deer and he and Sheather (1916), referred to it in buffalo, while Jarmai (1922) described a case in a White-bearded Gnu *Connochaetes albojubatus*. So far as the writers are aware, the disease has not been recorded in any other species.

This paper is an account of a condition which was almost certainly Johne's disease, occurring in a Llama (*Llama glama*).

DESCRIPTION OF CASE

History

The animal was one of a small herd in a ten acre enclosure at the Zoological Park, Edinburgh. It had been bred in captivity in this enclosure during the previous winter and was about fourteen months old at the time of death. A number of sheep shared the paddock and the grass was therefore very heavily grazed.

Symptoms were first observed on the morning of 18th January, 1950, when the animal was seen to be ' scouring ' badly. Two days later it became prostrate and too weak to rise but showed no sign of pain. Slight conjunctivitis was present. The animal was treated with "Thiazole" (M & B), but died on 24th January.

Post-mortem examination

Naked eye appearance. The carcase was dehydrated and emaciated. Significant lesions were restricted to the alimentary tract.

The stomachs contained a quantity of foodstuff and appeared normal except for slight flushing and petechiae of the abomasal mucosa.

In the small intestine, lymphoid patches were unusually prominent throughout. In the large intestine, the lymphoid aggregations varied between 0.5 cm. and 1.0 cm. in diameter and some showed a central depression with erosion. A number of nematode parasites resembling Trichuris species were found in the large intestine.

*At the time when this case was studied, E. C. Appleby was the holder of a Research Fellowship of the Animal Health Trust.

The mesenteric lymph nodes were enlarged and the cut surface showed some oedema and follicular hyperplasia. The larger nodes showed a thickened capsule enclosing creamy white necrotic material.

Histological appearance. The normal architecture of the mesenteric lymph nodes was replaced by large epithelioid cells forming a loose syncytium. The mucosa of the small and large intestine was heavily infiltrated with epithelioid cells replacing to a large extent the normal glandular pattern. A few plasma cells and lymphocytes were also present in this region but no giant cells were observed. The submucosal connective tissue showed focal aggregations of epithelioid cells, possibly representing invaded lymphoid follicles.

Sections of the lymph node and intestine stained by Ziehl-Neelsen's method showed the epithelioid cells to be packed with acid alcohol-fast bacteria morphologically identical with *Mycobacterium johnei.*

DISCUSSION

It is unfortunate that the true nature of the acid alcohol-fast organisms demonstrated histologically was not determined biologically or culturally. The macroscopic and microscopic appearance of the intestinal lesions leaves little doubt that the condition was one of Johne's disease, particularly in view of the enormous number of organisms present. Necrosis of the mesenteric lymph nodes is not a typical finding in cattle and sheep affected with Johne's disease but caseation of the nodes is mentioned in several of the reported cases of the disease in goats. It is felt therefore, that this particular finding is not inconsistent with the diagnosis in this case.

The source of infection is presumed to have been the sheep inhabiting the same pasture, although no deaths from this disease have occurred among them.

Taylor (1951, 1953) has shown that two types of the organism *M. johnei* can cause Johne's disease in British sheep. One of these types is pigmented and produces lesions of an orange colour in both sheep and cattle. No such colouration was observed in the Llama and it may therefore be supposed that the causal organism was the classical or bovine type of *M. johnei.*

ACKNOWLEDGMENT

The writers are indebted to Dr. A. W. Taylor for his advice.

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PATHOLOGY OF BRACKEN POISONING IN CATTLE

By

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INTRODUCTION

In a previous paper (Naftalin and Cushnie, 1951) we indicated briefly our haematological and post-mortem findings in cattle which had died after being fed experimentally on bracken (*Pteris aquilina*). This paper deals with our detailed investigations.

EXPERIMENTAL PROCEDURES

Experiment 1. Began July 20th, 1950. Twelve Ayrshire heifers were allocated into 3 groups of 4; group 1 received 60 per cent. of its feed as sun dried bracken and 40 per cent. hay by weight; group 2, 60 per cent. sun dried bracken, 20 per cent. hay and 20 per cent. molasses; and group 3, 60 per cent. grass and 40 per cent. hay (control group). The bracken was cut by scythe in July, 1950 and spread out in the sun to dry. It was chopped in a hay cutter before feeding and introduced into the diet in stages; 20 per cent. for 4 days; 40 per cent. for 4 days; finally it formed 60 per cent. of the diet.

Experiment 2. Began 5th May, 1951. In this experiment, set up to study the effect of members of B group of vitamins given parenterally, four Ayrshire heifers were used.

Heifer 1, age 10 months, weight 5 cwt. 2 qr. 0 lb. From the beginning of the experiment the following vitamins were given subcutaneously daily in 20ml. of distilled water, aneurin 50 mg., riboflavin 30 mg., pyridoxin 30 mg., calcium pantothenate 200 mg., biotin 2 mg., folic acid 30 mg., inositol 200 mg., nicotinamide 400 mg.

Heifer 2, age 10 months, weight 5 cwt. 1 qr. 2 lb. was given 50 mg. aneurin subcutaneously daily in 20ml. distilled water from 5th May till 30th July. On 31st July the dose was increased to 200 mg. daily and from 2nd August till 5th August 250 mg. twice daily doses were given.

Heifer 3, age 20 months, weight 7 cwt., and heifer 4, age 20 months, weight 6 cwt. 3 qr. 11 lb., were not given any vitamin supplement.

Feeding. Heifers 1, 2 and 3 were fed on 60 per cent. sun dried bracken and 40 per cent. hay from 5th May till 5th July. The bracken used had been harvested and dried in July, 1950 and stored. It was chopped immediately before feeding. From 6th July till the end of the experiment chopped fresh green bracken only was fed, the hay being omitted. Heifer 4 was fed on dried grass 60 per cent. and hay 40 per cent. till 5th July, when hay only was fed.

In both experiments daily observations were made of the amount of food eaten. Drinking water was always available but the amount drunk was not measured. Rectal temperature and heart rate were observed daily. The animals were weighed weekly. Examinations of urine and faeces for blood were carried out frequently.

Bacteriological and haematological investigations. In experiment 2 venous

blood was withdrawn from each heifer at weekly intervals till the temperature rose, after which more frequent withdrawals were made.

Methods. (a) Bacteriological. The skin over the external jugular vein was shaved and swabbed with an alcoholic solution of iodine. The apparatus used to remove the blood consisted of a 2 inch 14 B.S.W.G. needle connected to about 18 inches of ¼-inch rubber tubing which in turn was attached by a glass tube to a 50 ml. conical flask containing 4 ml. of 3.8 per cent. sodium citrate solution. Through the stopper of the flask another glass tube, plugged with cotton wool, served as an air escape. The assembled apparatus was sterilised by autoclaving. Approximately 20 ml. of blood were run into the flask. By this means it was possible to obtain samples of blood uncontaminated by the air of the byre. In the laboratory 5 ml. of the blood citrate mixture were transferred to 100 ml. of Wright's meat broth and incubated for 7 days at 37°C.

(b) Haematological. For red cell, white cell, and platelet counts and the estimation of haemoglobin, blood was obtained by removing the rubber tubing from the needle still in the jugular vein and attaching a 20 ml. sterilised syringe. Five ml. of blood were put into a 30 ml. bottle containing Wintrobe's dried oxalate mixture (6 mg. of ammonium oxalate and 4 mg. of potassium oxalate). The method of counting the platelets was that described by Baar (1948). Haemoglobin was estimated by Haldane's method. The degree of clot retraction was determined as follows: 10 ml. of venous blood were delivered into a graduated centrifuge tube. A copper wire, bent to a hook at its lower end, passed from the cork almost to the bottom of the tube. After 2 hours at 37°C. the cork with attached copper wire and adherent clot was removed, and the amount of serum expressed was noted.

RESULTS

The results were substantially the same in both experiments. All the animals fed on bracken lost condition and eventually died. Their skin became slack, lost its lustre, and the hair thinned. Hollows appeared in front of and behind the iliac crests. Molasses added to the bracken-hay mixture made no difference to the development of the disease (Table I).

An early sign in heifers 1 and 3 (experiment 2) was the presence of large numbers of squamous epithelial cells in the urine. These cells were observed on 7th and 13th July, i.e., 9 and 14 days before red blood cells were found in the urine. In heifer 2 epithelial cells and red blood cells appeared on 20th June.

Heart rates: rectal temperature. In experiment 1, the heart rates fell from initial rates of 60 to 70 beats a minute to 40 to 50 beats a minute in about 10 days after the commencement of feeding with bracken and remained slow until the temperature rose to 104°F. or more. In heifer 1 of experiment 2 the rate varied between 58 and 74 beats a minute until the temperature rose to 107.2°F. on 2nd August when it increased to 107. In heifer 2 the rate was 58 at the beginning of the experiment, fell to about 40 from 16th to 20th July, after which it returned to about 58. On the last days of its life the rate

TABLE I

SURVIVAL TIME (DAYS) OF HEIFERS FROM START OF FEEDING WITH BRACKEN

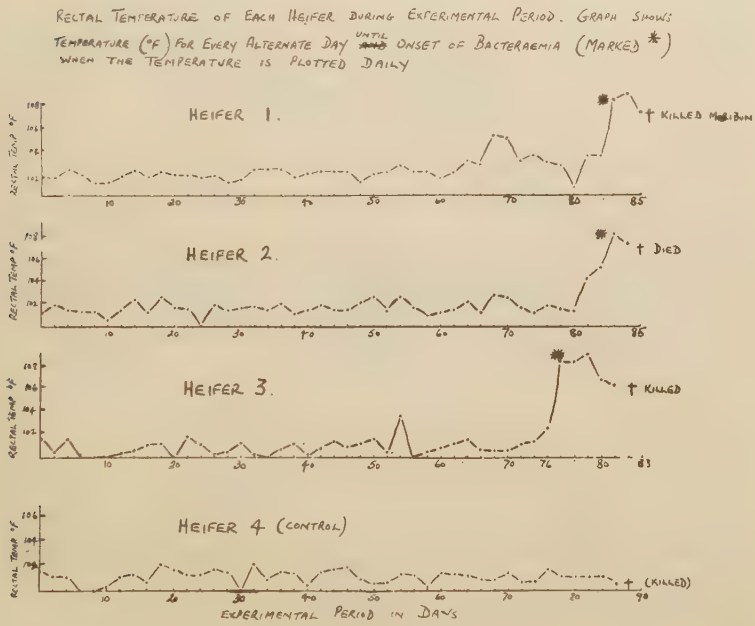
Expt. No.	*Heifer no.*	*Fed on*	*Days eating bracken before rise in temperature*	*Days lived after rise in temperature*	*Total survival time*	*Date of death*	*Remarks*
1	22749	Bracken and hay	32	6	38	28/8/50	Typical bracken poisoning. Subcapsular kidney haemorrhages (Fig. 13). Liver infarcts. Gelatinous tissue in mediastinum.
	22723	Bracken, hay and molasses	32	8	40	30/8/50	Typical bracken poisoning. Jaundiced. Liver infarcts. No haemorrhage in rumen or omasum.
	22733	Bracken and hay	33	7	40	30/8/50	Typical bracken poisoning.
	87121	Bracken and hay	33	7	45	4/9/50	Typical bracken poisoning. Rumen normal. Abscess in anterior abdominal wall.
	66N	Bracken, hay and molasses	53	4	57	16/9/50	Typical bracken poisoning. Had transient rise in temperature up to 105° F. from 38-41st day.
	22730	Bracken, hay and molasses	56	4	60	18/9/50	Typical bracken poisoning. Bacterial infarcts in kidney. Had transient rise in temperature up to 105°F. from 31-33rd day.
	30N	Bracken, hay and molasses	58	3	61	20/9/50	Typical bracken poisoning.
	22726	Bracken and hay	59	3	62	21/9/50	Typical bracken poisoning.
2	1	Bracken and hay (Given 8 vitamins of B group* parenterally)	86	5	91	4/8/51	Typical bracken poisoning. Shot when moribund. Multiple pale infarcts in liver (Fig. 11).
	2	Bracken and hay (Given vitamin B_1 parenterally)	86	6	92	5/8/51	Died of bracken poisoning. Multiple liver infarcts.
	3	Bracken and hay	81	5	86	30/7/51	Typical bracken poisoning. Shot when moribund. Multiple red infarcts in liver (Fig. 12).
	4	Grass and hay (control)	..	..	..	22/8/51	Remained well. Killed for comparison of tissues.

*From the beginning of the experiment the following vitamins were given subcutaneously daily in 20 ml. of distilled water; aneurin 50 mg., riboflavin 30 mg., pyridoxin 30 m.g. calcium pantothenate 200 mg., biotin 2 mg., folic acid 30 mg., inositol 200 mg. nicotinamide 400 mg.

rose to 88 to 116 beats a minute. Heifer 3 had a slower rate of 40 to 44 except in the five days preceding its death when it was 76 to 90 beats a minute. Again the rise in rate in heifers 2 and 3 coincided with a rise in rectal temperature.

It will be noted that a rise in temperature to 105 to 107°F. was coincident with bacteraemia (Fig. 1, expt. 2).

Fig. 1.


RECTAL TEMPERATURE OF EACH HEIFER DURING EXPERIMENTAL PERIOD. GRAPH SHOWS TEMPERATURE (°F) FOR EVERY ALTERNATE DAY UNTIL ONSET OF BACTERAEMIA (MARKED *) WHEN THE TEMPERATURE IS PLOTTED DAILY
HEIFER 1.
† KILLED MORIBUN
HEIFER 2.
† DIED
HEIFER 3.
† KILLED
HEIFER 4 (CONTROL)
† (KILLED)
EXPERIMENTAL PERIOD IN DAYS

Amount of food eaten. In both experiments the heifers fed on bracken ate the ration reasonably well, but rarely completed each day's feed. Fig. 2 shows the total weekly intake of food for heifers 1, 2, 3 and 4 (experiment 2). It will be seen that heifers 1, 2 and 3 ate between 50 and 70 lb. weekly of the hay-dried bracken mixture at the beginning of the experiment, rising to 80 to 90 lb./week after 9 weeks. The consumption of fresh bracken was more variable, appetite falling on two occasions in heifers 1 and 2. Heifer 4 was given 15 lb. of food a day (105 lb./week).

Weights. (Fig. 3). Heifers 1 and 2 of experiment 2 maintained their weight or increased slightly during the first 9 weeks of the experiment when their diet was dried bracken and hay; heifer 3 after an initial loss of about 50 lb. also maintained its weight but when fresh green bracken only was fed all three animals lost weight. The control heifer 4 gained in weight throughout the experiment.

Haematology. Some of the haematological results of experiment 1

Fig. 2

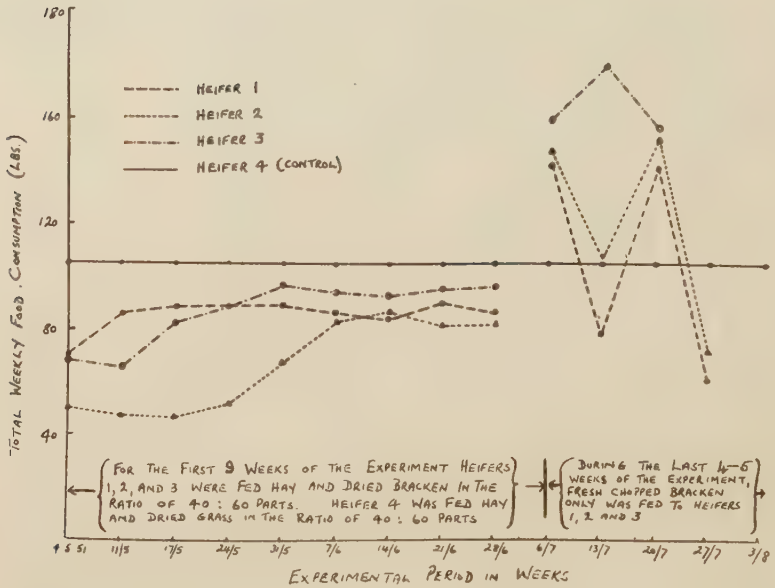
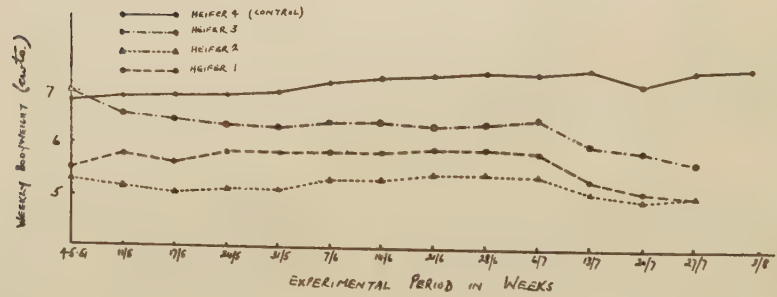

180
160
120
80
40
HEIFER 1
HEIFER 2
HEIFER 3
HEIFER 4 (CONTROL)
TOTAL WEEKLY FOOD CONSUMPTION (LBS.)
FOR THE FIRST 9 WEEKS OF THE EXPERIMENT HEIFERS 1, 2, AND 3 WERE FED HAY AND DRIED BRACKEN IN THE RATIO OF 40 : 60 PARTS. HEIFER 4 WAS FED HAY AND DRIED GRASS IN THE RATIO OF 40 : 60 PARTS
DURING THE LAST 4–6 WEEKS OF THE EXPERIMENT, FRESH CHOPPED BRACKEN ONLY WAS FED TO HEIFERS 1, 2 AND 3
4 5 51 11/5 17/5 24/5 31/5 7/6 14/6 21/6 28/6 6/7 13/7 20/7 27/7 3/8
EXPERIMENTAL PERIOD IN WEEKS

Fig. 3



have already been published (Naftalin and Cushnie, 1951). As more complete data were obtained from experiment 2, these only are presented (Tables II & III and Fig. 4, 5, 6 and 7). The main features were a progressive diminution of the number of white cells and platelets per c.mm. In heifers 1 and 3 the number of platelets increased to normal values during the seventh and eighth weeks of the experiment and in heifer 1 the white cells increased to 15,400 during the 9th week (Figs, 4 & 5). After the 26th July (12th week of experiment 2), when the platelet counts fell to about 40,000 per c.mm. or lower, the clot failed to retract when the blood was incubated at 37°C. for two hours (Table II).

Fig. 4

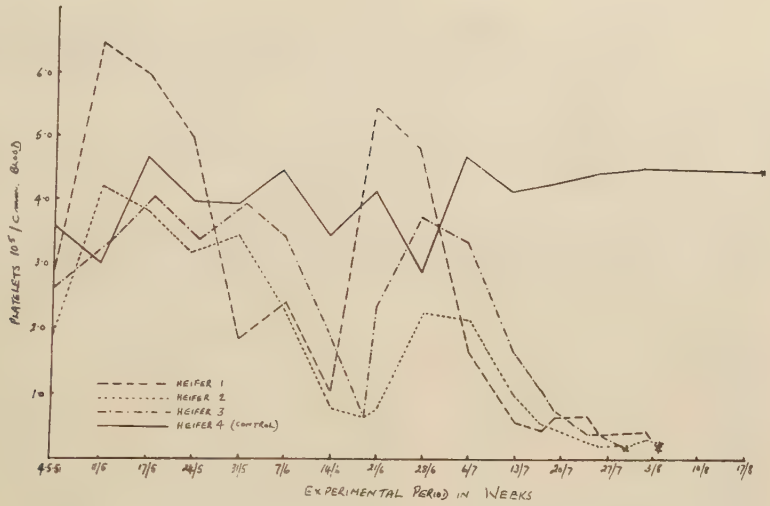
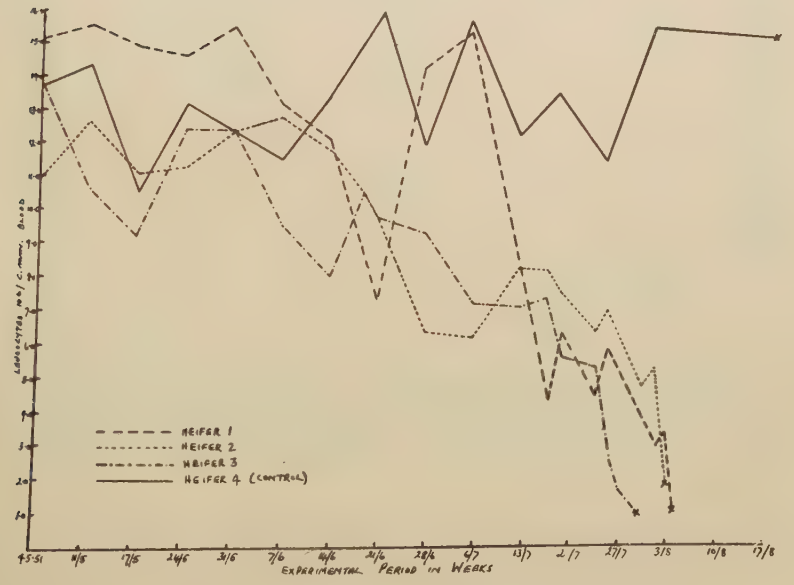


Fig. 5



Bacteriology. The results of the blood cultures and post-mortem bacteriological investigations (experiment 2) are summarised in Table IV.

Deaths. (Table I). All the animals fed on bracken in experiment 1 died, with signs of bracken poisoning. Death was due to extensive

TABLE II

SERIAL DIFFERENTIAL LEUCOCYTE COUNTS IN

Heifers Nos. 1, 2 and 3 fed bracken in diet from 5/5/51,

	HEIFER no. 1						*HEIFER no. 2*					
	Differential leucocyte count %						*Differential leucocyte count %*					
Date	*Poly-morphs*	*Meta-myelocytes*	*Eosino-philes*	*Baso-philes*	*Mono-cytes*	*Lympho-cytes*	*Poly-morphs*	*Meta-myelocytes*	*Eosino-philes*	*Baso-philes*	*Mono-cytes*	*Lympho-cytes*
3.5.51	..	..	..	..	..	..	..	..	..	..	..	..
4.5.51	24	2	4	0	4.5	65.5	28.5	0	6.5	0	4	61
11.5.51	17	1	4	0	3	75	28	0	5	0	4	63
17.5.51	16	1	8	0	7	68	10	1	5	0	6	78
24.5.51	15	0	3	0	9	73	16	0	5	0	6	73
31.5.51	11	0	8	0	7	74	22	0	8	0	4	66
7.6.51	36	1	0	0	4	59	12	0	5	0	4	79
14.6.51	17	0	1	0	1	81	16	3	5	0	6	70
19.6.51	..	..	..	..	..	..	..	..	..	..	..	..
21.6.51	3	0	1	0	4	92	12	0	3	1	2	82
28.6.51	32	5	0	0	3	60	2	0	0	0	1	93
6.7.51	25	1	6	0	1	67	8	0	3	0	2	87
13.7.51	4	0	1	0	0	95	8	0	0	1	1	90
17.7.51	1	3	0	0	1	95	13	1	0	0	0	86
19.7.51	1	2	0	0	0	97	4	0	1	0	1	94
24.7.51	3	3	0	0	1	93	3	1	2	0	1	93
26.7.51	8	3	0	1	1	87	1	0	1	0	1	97
27.7.51	..	..	..	..	..	..	..	..	..	..	..	..
28.7.51	..	..	..	..	..	..	..	..	..	..	..	..
30.7.51	..	..	..	..	..	..	..	..	..	..	..	..
31.7.51	..	..	..	..	..	..	2	4	0	0	0	94
2.8.51	0	3	0	0	0	97	0	2	0	0	1	97
3.8.51	0	0	0	0	0	100	0	0	0	0	0	100
4.8.51	0	0	0	0	0	100	..	..	..	..	..	..
5.8.51	..	..	..	..	..	..	0	0	0	0	0	100
20.8.51	† 4.8.51						† 5.8.51					

†Killed or died.
*Not measurable, clot friable.

internal haemorrhage. In experiment 2, heifers 1 and 3 were shot by captive-bolt-pistol when moribund. Heifer 2 died suddenly from internal haemorrhage. Heifer 4 was shot and its tissues studied for comparison. The time from first feeding the bracken till death is also shown in Table I.

Post-mortem Findings

The main feature was multiple haemorrhages throughout the carcase, their degree and exact location varying from animal to animal. Haemorrhages were observed in subcutaneous tissues, in the fascial planes, into the muscles, serosae, subserosae, mucosae and submucosae. The subcutaneous tissue of the neck of three heifers in experiment 1 and of heifer 1 of experiment 2, was grossly oedematous and gelatinous in appearance (Fig. 8). The mediastinum

PERIPHERAL BLOOD AND DEGREE OF CLOT RETRACTION
and of heifer No. 4 fed on hay and grass; experiment 2

HEIFER no. 3						*HEIFER no. 4*						*Degree of clot retraction. Serum expressed after incubation 2 hr. at 37°C. as per cent. of initial volume (5 ml.) of blood.*			
Differential leucocyte count %						*Differential leucocyte count %*									
Poly-morphs	*Meta-myelocytes*	*Eosino-philes*	*Baso-philes*	*Mono-cytes*	*Lympho-cytes*	*Poly-morphs*	*Meta-myelocytes*	*Eosino-philes*	*Baso-philes*	*Mono-cytes*	*Lympho-cytes*	*Heifer no. 1*	*Heifer no. 2*	*Heifer no. 3*	*Heifer no. 4*
..	..	..	..	..	..	..	..	..	..	..	..	60	56	..	..
32	1	4·5	0	2·5	60	25·5	1	4	0	6	63·5	55	55	40	54
21	1	7	0	1	70	27	0	5	5	5	63	60	40	56	54
11	1	12	0	5	71	12	1	11	0	7	69	66	56	48	48
10	0	10	1	5	74	16	0	11	0	5	68	60	56	44	42
14	0	7	0	9	70	16	1	8	0	11	64	72	62	58	54
10	0	12	2	6	70	19	0	8	0	8	65	48	*	*	50
20	0	16	0	1	63	15	0	5	0	8	72	..	..	..	..
7	0	8	0	3	82	..	..	..	..	..	..	..	..	..	..
5	0	10	0	2	83	19	1	4	0	4	72	56	56	40	50
5	2	2	0	2	89	8	0	8	0	6	78	70	56	72	72
11	0	3	0	3	83	15	0	5	0	6	74	48	42	54	52
14	0	4	0	0	82	15	2	3	0	11	69	34	38	52	56
22	0	3	1	0	74	..	..	..	..	..	..	..	..	..	..
10	0	7	1	0	82	21	0	4	1	7	67	40	40	40	54
1	0	0	0	0	99	..	..	..	..	..	..	..	..	..	..
1	0	0	0	1	98	16	0	8	0	5	71	0	0	0	58
0	0	0	0	0	100	..	..	..	..	..	..	..	..	..	..
0	1	0	0	0	99	..	..	..	..	..	..	0	0	0	56
1	0	0	0	0	99	..	..	..	..	..	..	..	..	..	..
						..	..	..	..	..	..	..	..	0†	..
						..	..	..	..	..	..	..	..		..
						8	0	11	1	6	74	..	0		..
						..	..	..	..	..	..	..	0		56
						..	..	..	..	..	..	0†	..		..
		†				..	..	..	..	..	..		0†		..
		30.7.51				17	0	3	0	7	73				58
								† 20.8.51							†

of one heifer in experiment 1 had a similar gelatinous appearance. Though they lost weight the animals were not very emaciated, subcutaneous and omental fat being present. Some of the organs are described in more detail.

Rumen. Multiple subepithelial and subserosal haemorrhages from pin-point size to 3 cm. in diameter were observed (Fig. 9). Section of the smaller lesions showed that the haemorrhage was punctuate under the mucosa and extended up to the epithelial layer (Fig 10). In the centre of the larger subepithelial haemorrhages the epithelium had sloughed, leaving a black base (Fig. 9). Areas of oedema were scattered irregularly throughout the rumen.

In the remainder of the alimentary tract from reticulum to rectum there were similar haemorrhages, ulcerations and oedema

Fig. 6

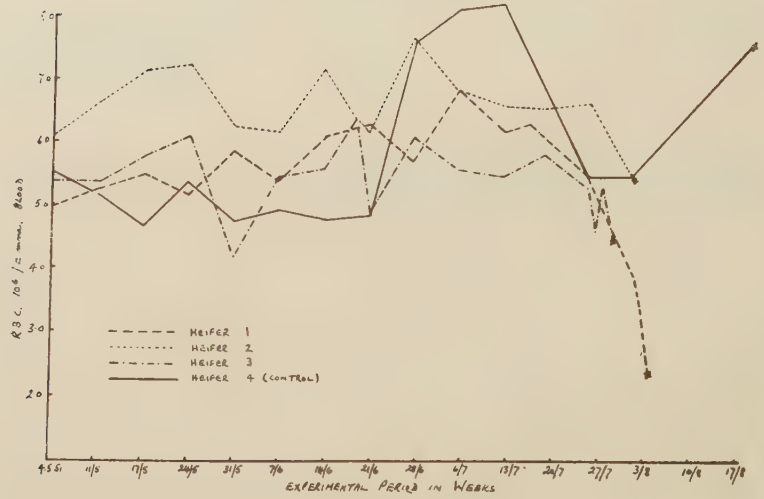
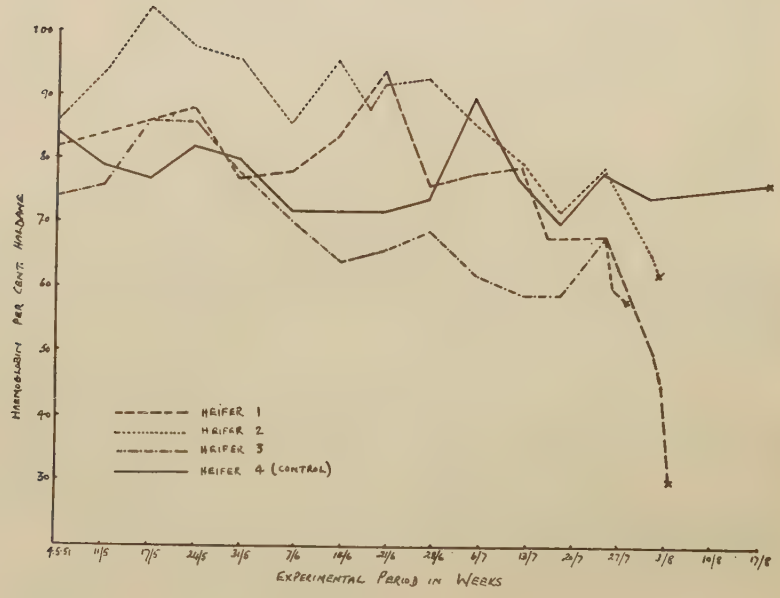

R.B.C. 10^6 / c.mm. Blood
HEIFER 1
HEIFER 2
HEIFER 3
HEIFER 4 (CONTROL)
EXPERIMENTAL PERIOD IN WEEKS

Fig. 7


HAEMOGLOBIN PER CENT. HALDANE
HEIFER 1
HEIFER 2
HEIFER 3
HEIFER 4 (CONTROL)
EXPERIMENTAL PERIOD IN WEEKS

as in the rumen, but in addition punctate haemorrhages were present in the mucosa as well as in the submucosa. Atrophy of all layers was observed in the small intestine in heifer 3 of experiment 2.

Liver. In experiment 1, the whole of each liver was not available for study. Grossly, small white areas about 0.5 cm. in diameter were noted in the liver of two heifers (22749 and 22723, Table I). In the three heifers fed on bracken in experiment 2, lesions measuring up to 1 cm. in diameter, were present in all lobes throughout the substance and under the capsule. The subcapsular lesions tended to be triangular in section. In heifers 1 and 2 these were yellow or whitish in colour, some uniform, others with lighter and darker areas (Fig. 11). In heifer 3 the lesions were red in colour and often near a portal vein (Fig. 12).

Gall bladder. The main feature was multiple subserosal and subepithelial haemorrhages. In heifer 1 of experiment 2 the intra and extrahepatic bile ducts were intensely haemorrhagic and oedematous.

Kidneys. (Fig. 13). In two heifers in experiment 1, small wedge-shaped haemorrhages were noted under the capsule. This lesion was not observed in the three heifers fed on bracken of experiment 2 where macroscopically the kidneys appeared normal.

TABLE III

DIFFERENTIAL COUNTS PER CENT OF BONE MARROW CELLS

Obtained from sternum immediately after each animal was killed or died

	Heifer 1	*Heifer 2*	*Heifer 3*	*Heifer 4*	*Average cell count on bone marrow obtained from 6 cows or heifers at local slaughter-house*
Haemocytoblasts	0	0	0	0.25	0.33
Normoblasts					
Early	1.5	1	0	1.0	1.12
Intermediate	16.5	10.0	18.0	14.0	12.75
Late	1.5	4.0	12.0	29.5	41.16
Blast cells	0	0	1	2	0.87
Promyelocytes	0.25	0	1	1	1.16
Myelocytes	1	0	0	3.25	1.41
Metamyelocytes	1	0	1	11.25	4.66
Polymorphonuclear neutrophiles	0	0	1	22.25	19.91
Eosinophiles	2.25	0	0	5.5	10.29
Basophiles	0	0	0	0	0
Monocytes	0	2	0	0.5	0.87
Lymphocytes	75.5	77	66	6	4.04
Plasma cells	0	2.0	0	0	0.04
Megakaryocytes	0	0	0	0.25	0.50
Other cells*	0.5	4	0	0.25	0.42
Myeloid/erythroid ratio†	0.23	..	0.13	1.04	0.69

*In this cell group are included mast cells, reticulo-endothelial cells, and stroma cells.

†It was impossible to calculate the Myeloid/Erythroid ratio for heifer 2 because no cell of the myeloid series was found in the marrow smears examined.

TABLE IV

BACTERIOLOGICAL

Experiment 2, in

	Blood cultures			
	11th May—26th July 11 cultures	*27th July*	*30th July*	*2nd Aug.*
Heifer no. 1	No growth	..	..	Staph. albus
Heifer no. 2	No growth	..	..	Micrococcus sp.
Heifer no. 3	No growth	Strep. dysgalactiae	Strep. dysgalactiae B. coli (Heifer killed)	..
Heifer no. 4	No growth	..	..	..

Ureters and bladder. In every heifer fed on bracken there were multiple submucosal haemorrhages extending throughout the urinary tract from the calyces of the kidneys to the urethra. The degree of haemorrhage varied from pin-point lesions to confluent haemorrhages with almost complete infiltration of the submucosa. There was no obvious gross ulceration.

Heart. Subepicardial and subendocardial haemorrhages varying in size from pin-point to several centimeters in diameter were present. Yellow areas up to 1 cm. in size were seen on both the exterior and interior surfaces.

Lungs. In experiment 1, three heifers had large haemorrhages scattered throughout the lungs. In experiment 2 a few pin-point haemorrhages were observed in heifers 1 and 3 only.

Similar haemorrhages were noted in the nasal mucosa, tongue, larynx and trachea, in the pancreas, in the capsule of the spleen but not in its substance, under the capsule and in the hila of lymph glands, under the capsules of the adrenal glands and thyroid, in the mammary gland, ovaries, uterus and cervix, the aorta, externally and internally, and in the dura mater and under the pia mater; no haemorrhages were seen in the brain itself. There were no haemorrhages into the joint cavities.

Bone marrow. The sternal bone marrow was examined within a few minutes of death in heifers 1 and 3, and after about 1 hour in heifer 2. The marrow was pale red, in contrast to the deep red coloured marrow of normal cattle.

INVESTIGATIONS

heifers 1, 2, 3 and 4

			Post mortem bacteriological investigations			
3rd Aug.	*4th Aug.*	*5th Aug.*	*Heart blood*	*Liver*	*Kidney*	*Spleen*
Pasteurella sp.	Pasteurella sp. (Heifer killed)		Pasteurella sp.	Pasteurella sp.	No growth	No growth
Pasteurella sp.	Pasteurella sp.	Heifer died	..	Pasteurella sp. B. coli	No growth	No growth
..	..	..	B. coli	Strep. dysgalactiae	No growth	No growth
..	..	..	No growth	No growth	No growth	No growth

Histology

Microscopically, the haemorrhages were of two types, (1) around the smaller arterioles and the capillaries, i.e., the type associated with thrombocytopenia (Fig. 14); (2) haemorrhages associated with bacterial infarction in the liver, kidney, lung and muscle (Figs. 23, 24, 25, 26, 27, 28, 35).

Bone marrow (sternum) (Figs. 15 and 16, and Table II). There was a marked diminution of cells in the bone marrow. The degree of cell loss varied from area to area, little islets of marrow tissue being observed in some areas, whereas in others there was only a fine oedematous stroma containing a few cells and fat. The capillaries were dilated. Many small haemorrhages were present in the stroma. Megakaryocytes were not seen in smears nor in many sections examined.

Rumen. Haemorrhages were consistently observed in the submucosa and subserosa occasionally between the muscle bundles. The submucosa and subserosa were often oedematous and in some areas infiltrated with mononuclear and plasma cells, while fibroblastic proliferation was seen in some sections. Many of the smaller vessels had a collar of mononuclear and plasma cells (Fig. 17 and 19). Occasionally swollen endothelial cells were seen in capillaries (Fig. 20). The muscle bundles were atrophied and frequently fragmented, being separated by oedematous tissue containing inflammatory cells (Fig. 18). Small areas of necrosis, up to 600 μ in size, were scattered throughout the submucosal collagen. These areas of necrosis sometimes contained bacteria.

Overlying the ulcers were dense masses of bacteria which occasionally penetrated into the viable tissue beneath, where the thin walled blood vessels often contained deposits of fibrin. Occasionally bacteria were seen in these vessels but usually the bacterial masses lay outside. In the submucosa of the rumen of heifer 2 experiment 2, a gram positive fungus was observed among the inflammatory cells.

Alimentary tract. Lesions similar to those described in the rumen were noted throughout the remainder of the alimentary tract.

Liver. The following lesions were observed: centrilobular necrosis (4 heifers) (Fig. 21); focal necrosis (7 heifers) (Fig. 22); areas of fatty change under the capsule (heifer 1 of experiment 2); haemorrhagic or ischaemic infarcts associated with bacteria or fibrin (Figs. 23, 24, 25), and occasionally infiltration with small round cells and mononuclear cells in the portal triad particularly in relation to the infarcts. No fibrosis was found.

Liver infarcts. The white and red areas were found to consist of necrotic liver tissue with large numbers of bacteria or deposits of fibrin or both in the smaller branches of the portal vein, in the sinusoids, and in the central vein. The red areas had numerous red blood cells in the lesions. The bacteria in the sinusoids often seemed to form a ring enclosing a small area of liver. Fibrinoid degeneration of the hepatic artery in the portal triad was seen in some of the larger infarcts. The sinusoids within the infarcted area were dilated. In some sections bacteria were present in small clumps in the sinusoids and central veins without causing infarction (Fig. 21).

Kidney. The wedge-shaped haemorrhagic areas seen under the capsule were bacterial infarcts (Figs. 13 and 26). Bacteria, usually gram positive cocci, were present in masses in the periglomerular capillaries surrounding necrotic tubules (Fig. 27) within Bowman's capsule (Fig. 28) and in the tubules in the medulla. It should be noted that the tissues around these structures had reacted—indicative of an ante-mortem phenomenon.

Scattered throughout the kindeys of three heifers of experiment 1 were perivascular haemorrhages of the type associated with thrombocytopenia (Fig. 14). Perivascular mononuclear and round-cell infiltrations were present in two of these kidneys (Fig. 30). In experiment 1 in three kidneys not showing bacterial infarction, eosinophilic material was observed within Bowman's capsule (Fig. 29).

Bladder. The epithelium showed degenerative changes. Vacuolation of the cells, or desquamation leaving an exposed basement membrane was a frequent finding (Figs. 31, 32 and 33). The basement membrane appeared to remain intact. Many small haemorrhages were present in the oedematous submucosa. Plasma cells, mononuclear cells and large phagocytes containing an erythro-

cyte or haemosiderin were scattered throughout the submucosa. The cellular infiltration was not as dense as in the alimentary tract. The capillaries occasionally had a collar of plasma cells or mononuclear cells.

Lungs. In the lungs with extensive haemorrhage, bacterial infarction, in addition to purpuric lesions, was undoubtedly present though it was difficult to assess the relative importance of each type of lesion. Clumps of gram-positive and gram-negative bacilli appeared in some instances to occlude the small vessels (Figs. 34 and 35). Perivascular haemorrhage, particularly affecting the smaller arterioles, was a frequent finding in these lungs. There was massive infiltration with r.b.c. of the alveoli and interseptal spaces while at the periphery of a haemorrhagic area there was marked oedema.

Thyroid, adrenal and pituitary glands. Apart from occasional haemorrhage into the capsules of the thyroids and adrenal glands, no histological changes were noted in haematoxylin eosin stained sections of these three glands.

DISCUSSION

It is apparent from the above description that the essential lesion in bracken poisoning in cattle is bone marrow damage A diminution in the numbers of circulating platelets and granulocytes results. Subsequently there are multiple haemorrhages and bacterial invasion of the blood stream. Bacterial invasion seems to take place through the gut by the following mechanism: haemorrhage into the mucosa or submucosa is followed by ulceration. Bacteria multiply on the surface or in the necrotic tissue, and then, singly or in clumps, enter the mesenteric and portal vessels, are carried to the liver, where they may lodge, or pass through into the general circulation. Infarction of the liver does not seem to take place unless a small portal vein and the surrounding sinusoids are blocked, thus forming a ring of ischaemic tissue.

In experiment 1, where heifer 22730 and heifer 22749 died 4 and 6 days after the rise in rectal temperature to 105 to 107°F., haemorrhagic infarction due to bacteria was also present in the kidneys and in the lungs. Infarction in the spleen was not noted in these animals. Some of the liver infarcts had a lymphocytic and mononuclear infiltration but no cellular inflammatory reaction was present in the haemorrhagic infarcts in the kidneys. This is in accordance with the severe polymorphonuclear leucopenia and with the probable longer duration of the liver infarcts. The infarcts in the kidneys of our animals had the appearance of subterminal phenomenon. Widespread coagulative necrosis without haemorrhage and without any inflammatory reaction has been described by Guilhon, Drieux, Gasse, Perdrix and Julou (1950) in the kidney of a seven month old calf which had been bedded continuously on bracken. They suggested that the lesion was a toxic one, resembling

experimental nephritis caused by mercury sublimate or uranium nitrate.

Apart from the bacterial infarctions the morbid anatomical picture was that of severe thrombocytopenic purpura and resembled the lesions produced in dogs by injection of anti-dog-platelet serum (Tocantins and Stewart, 1939). A similar morbid anatomical picture as the result of extensive bone marrow damage has been described by Sautter, Rehfeld and Pritchard (1952) in cattle which had been fed on trichloroethylene-extracted soyabean meal. (Stockman Disease, Duren Disease, Brabant Disease). Humble (1949) has shown that in thrombocytopenic purpura in man, bleeding takes place from the arterio-capillary junction, but from the study of sections we are unable to say exactly at what point bleeding took place. A feature of note in our cattle was the collar of haemorrhage in the smaller arterioles of the kidneys, bile ducts, the arterioles of the gut and of the lungs.

Haemorrhage became more severe after the onset of bacteraemia. The cause of death was extensive internal haemorrhage.

That bracken poisoning in cattle was associated with haematological changes (thrombocytopenia, leucupenia, defective blood clotting) has been reported by many workers (Schofield, 1947; Faulds (quoted by Robinson, 1947); Guilhon and Julou, 1949, 1950; Guilhon and Dradillat, 1950; Guilhon, 1950; Jacquet, 1950; Naftalin and Cushnie, 1951; Evans, Evans and Hughes, 1951; Osebold, 1951). A short account of the pathology is given by Guilhon, Drieux and Julou (1950) and Guilhon et alia, (1950). In addition to haemorrhage and ulceration of the gut, they described oedema of the liver sinusoids and liver degeneration, with no inflammatory reaction, and degeneration of the convoluted tubules of the kidney with granular debris in the lumina.

Despite low platelet counts of 103,000 per c.mm. in heifer 1 in experiment 2 on the 14th June, and of 60,000 per c.mm. in heifer 3 on the 19th June and again in both these heifers from the 13th July, till the end of the experiment (Fig. 4) bleeding was not apparent clinically till the 16th (heifer 1) and 27th July (heifer 3). From the 20th June, six days after the low platelet count of 78,000 was recorded, heifer 2 had persistent haematuria. In heifers 1 and 3, the numbers of platelets rose to normal values of 545,000 and 374,000 per c.mm. during the last week of June. An increase beyond 204,000 per c.mm. was not recorded in heifer 2. No explanation is offered for these remissions. The number of platelets was again reduced on 13th July, three days after fresh bracken was fed. A low platelet count is a feature of bracken poisoning in the horse (Evans, Evans and Roberts, 1951) though the syndrome is not a haemorrhagic one. Bedson (1922) following a suggestion by Nolf (1911) (quoted by Bedson) is of the opinion that even with a marked thrombocytopenia, haemorrhage does not result unless there is capillary damage. Macfarlane (1940) and Ackroyd (1949a and b) support this view.

Ackroyd (1949b) goes as far as to suggest the necessity for a third factor, possibly allergic in nature, to precipitate haemorrhage in some instances of thrombocytopenia associated with increased capillary permeability, e.g., in the purpura occurring after rubella or in sedormid sensitivity. In his guinea-pigs which had been treated with anti-platelet-serum, Bedson described enlargement of the capillary endothelial cells and oedema in the perivascular tissue. In our cattle, oedema was a feature of the submucosae; we observed occasionally swelling of the capillary endothelium but many of the small thin walled vessels in the gut and bladder had a collar of mononuclear and plasma cells (Fig. 17, 19 and 20). The significance of these changes is difficult to assess in the presence of extravasations of red blood cells in the tissue. The question whether there is primary capillary damage in bracken poisoning in addition to low platelet count is still undecided and requires to be investigated in more detail. The degree of clot retraction seemed to bear no relation to the number of platelets when these were more than 40,000 per c.mm.

From the sixth week of experiment 2, there was a reduction in the numbers per c.mm. of the white cells in the venous blood, the neutrophils, eosinophils and monocytes being principally affected, but in heifer 1 the numbers rose to their initial value during the ninth week. (Fig. 5 and Table II). This remission was not coincident with the rise in the numbers of platelets, but appeared about one week later. The reason for this may be slower maturation of the white cells during a remission. Terminally there was complete absence of polymorphonuclear leucocytes and a severe reduction in the number of lymphocytes. In cattle fed experimentally on bracken, Hughes, Evans and Evans (1951) found that after the percentage of polymorphs had fallen to a low level there may be a transient increase with or without an increase in the number of w.b.c. per c.mm. In a horse fed on bracken, Evans, Evans and Roberts (1951) observed eosinophilopenia and thrombocytopenia but they report an accompanying polymorphonuclear neutrophil leucocytosis.

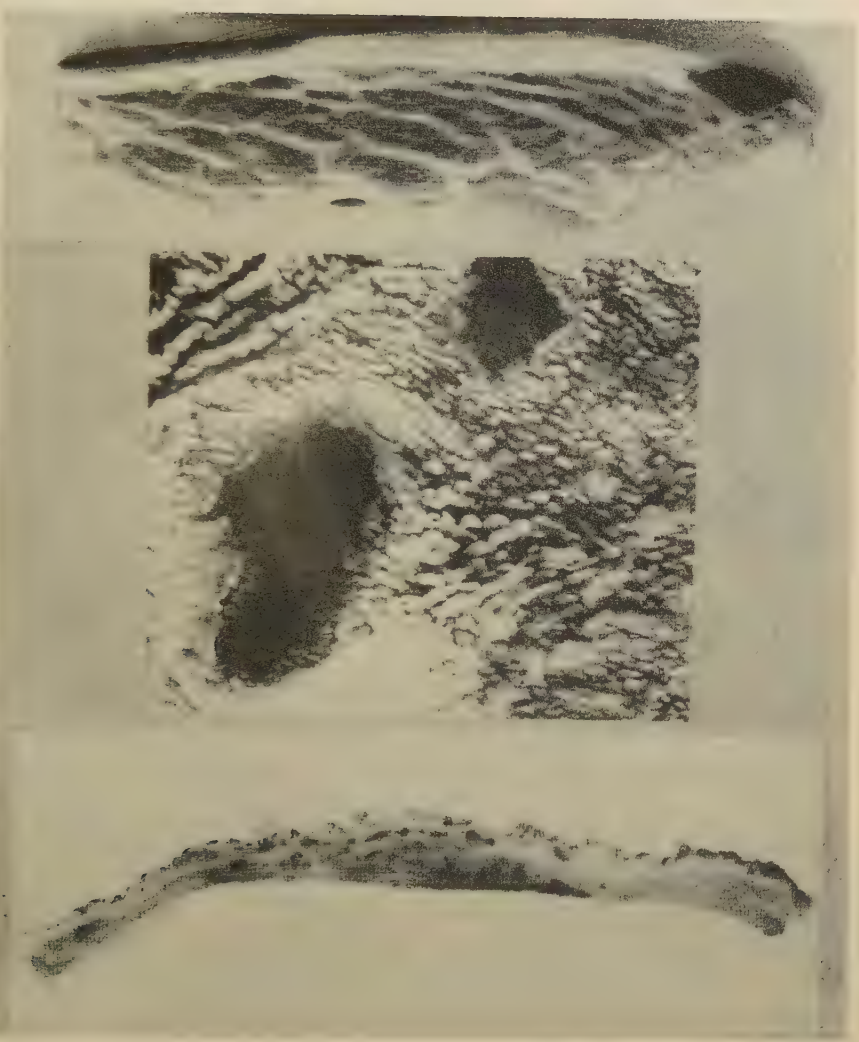
In contrast to the marked fall in numbers of platelets and white cells, the number of red blood cells per c.mm. remained within normal limits until the terminal severe bleeding (Fig. 6). The haemoglobin per cent showed a downward trend from the ninth week of experiment 2 (Fig. 7). However, examination of the bone marrow at death showed widespread destruction of the erythropoetic, as well as the leucopoetic, tissue. The circulating life of a red cell in man is estimated to be about 120 days (Shemin and Rittenberg, 1946; Callender, Powell and Witts, 1947; Ashby, 1948), that of a mature leucocyte only about 13 days after formation and 9 days after entering the circulation (Kline and Cliffton, 1952). This may account in part for the high figures for r.b.c. and Hb. throughout the experiment until severe bleeding took place.

When a non-ruminant, e.g., the horse or rat, eats a diet containing 40 per cent or more bracken, anorexia, loss of weight,

bradycardia and in-coordination of the hind limbs result. The syndrome is readily curable by the parenteral administration of vitamin B_1. Bracken contains an enzyme—thiaminase—which destroys vitamin B_1 (Weswig, Freed and Haag, 1946; Evans and Evans, 1949): hence the trial of vitamin B_1 in experiment 2. The other seven vitamins were tried because of a possible interference by bracken of their metabolism or of their production in the gut of a heifer fed on bracken. Experiment 2 shows that bracken poisoning was not prevented by giving parenterally from the beginning of the experiment either vitamin B_1 alone or vitamin B_1 together with seven other members of the B group of vitamins. When administered parenterally after the temperature had risen to 106°F. in the terminal stage of the disease, vitamin B_1 was found by Vaughan (1949), O'Moore (1949), Carpenter, Phillipson and Thomson 1950), Moore and Raafat (1951) and Wilson to be ineffective in preventing death. A similar result was obtained when vitamin B_1 was given prophylactically, by drench or parenterally (Hughes et alia; Wilson) and with multiple vitamins of the B group, including B_{12}, given parenterally, or nicotinamide by mouth (Hughes, et alia). Tissues of cattle dead of bracken poisoning do not have an appreciably lower vitamin B_1 content than normal (Hughes et alia) but Moon and Raafat, from their studies on bracken poisoning in sheep, concluded that although there is no evidence of acute B_1 deficiency there may be a subnormal vitamin B_1 status. Cattle suffering from bracken poisoning do not have paresis or any marked degree of anorexia, two of the classical signs of vitamin B_1 deficiency in non-ruminants. Vitamin B_1 deficiency, as such, however has not been described in adult ruminants. The explanation for the lack of B_1 deficiency signs in bracken-fed cattle may be that the ruminant derives its energy mainly from volatile fatty acids as a result of bacterial action in the rumen.

From his observations of a natural outbreak of bracken poisoning, McLauchlan (1951) thought that the ulcerative lesions of the tongue resembled clinically those of black tongue in dogs. He treated his 12 heifers with 600 mg. of nicotinamide by mouth daily for 2 to 3 days and reported recovery in all. However the histopathology of bracken poisoning in cattle does not resemble the description given by Denton (1925, 1928) of pellagra in man or of experimental black tongue in dogs. The initial lesion in these conditions is immediately below the epithelium and superficial to the subpapillary vascular plexus. The fine fibrillae which attach the epithelium to the stroma become fragmented. Secondary changes appear in the vessels and in the epithelium. The vessels become dilated, their walls thin, the endothelial cells long and slender; the epithelium desquamates. There is no mention of haemorrhage in Denton's account. Moreover the administration of nicotinamide parenterally in our experiment did not prevent bracken poisoning. McLauchlan's results are difficult to assess because occasionally

Fig. 8



g. 9

Fig. 10

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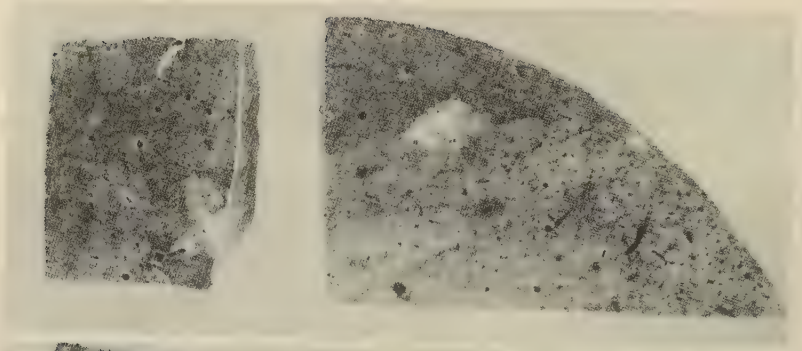

Fig. 11

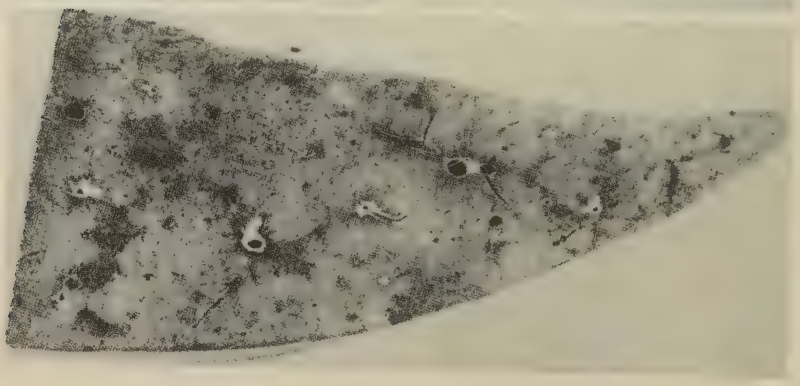

Fig. 12

Fig. 13

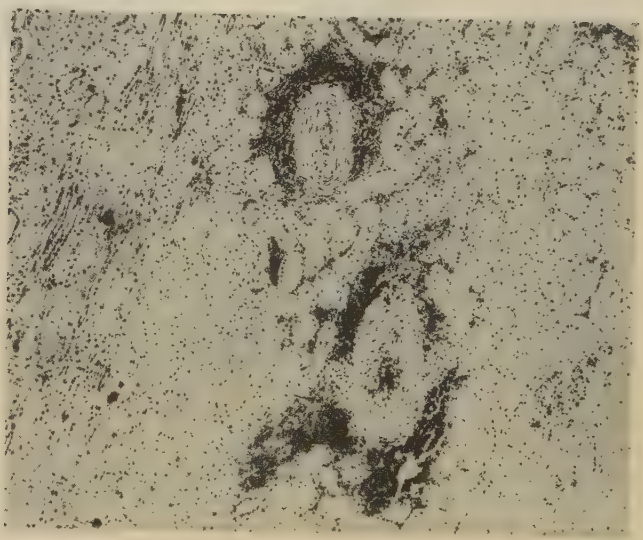

Fig. 14

Fig. 15

Fig. 16

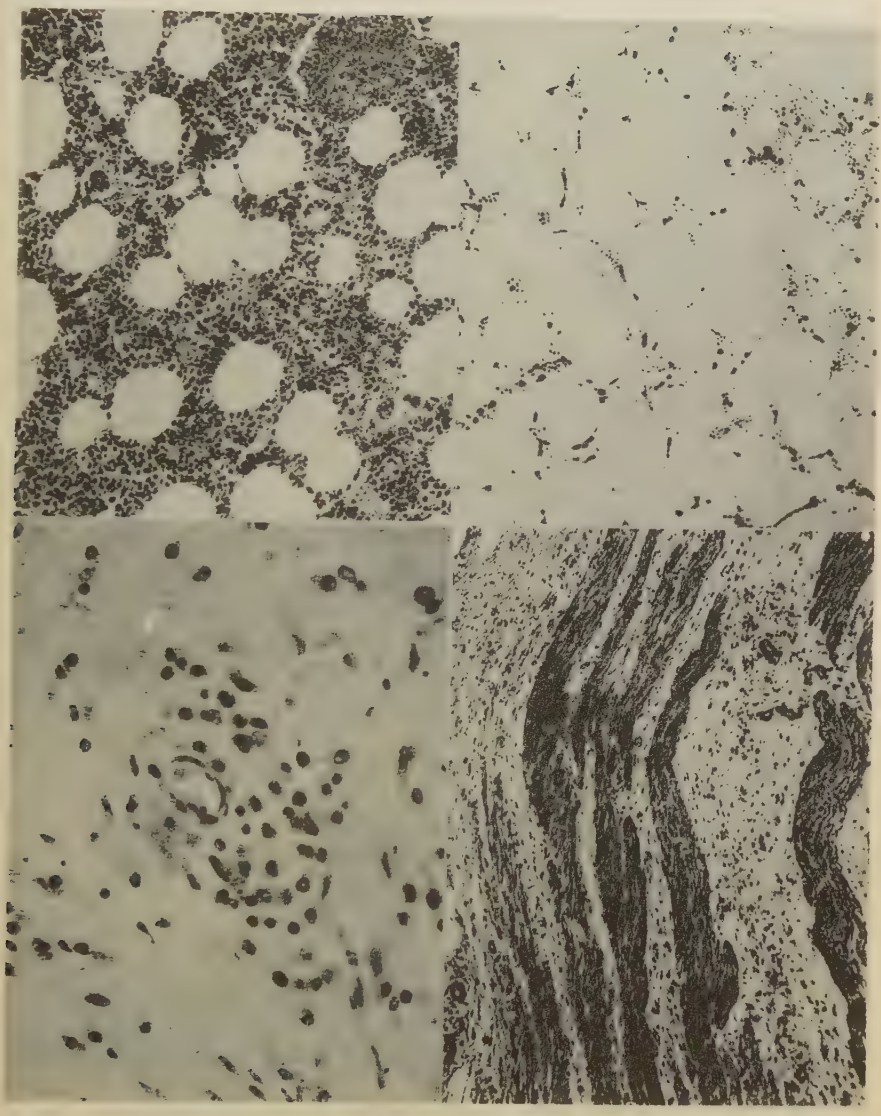

Fig. 17

Fig. 18

Fig. 19

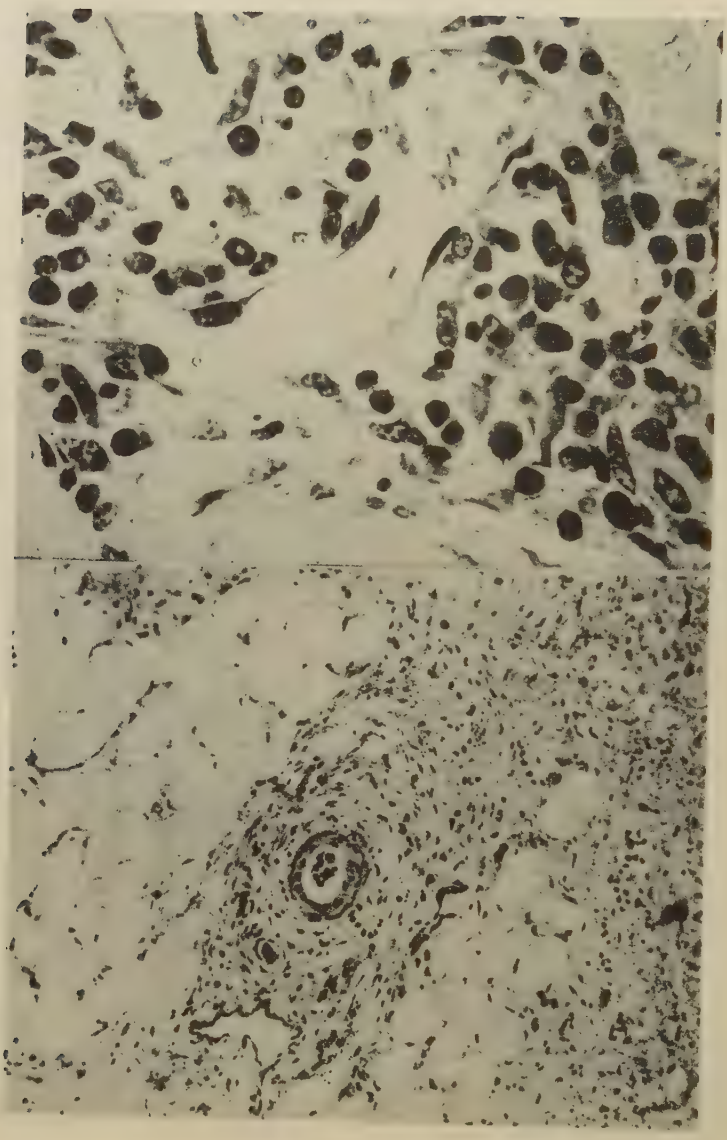

Fig. 20

Fig. 21.

Fig. 22

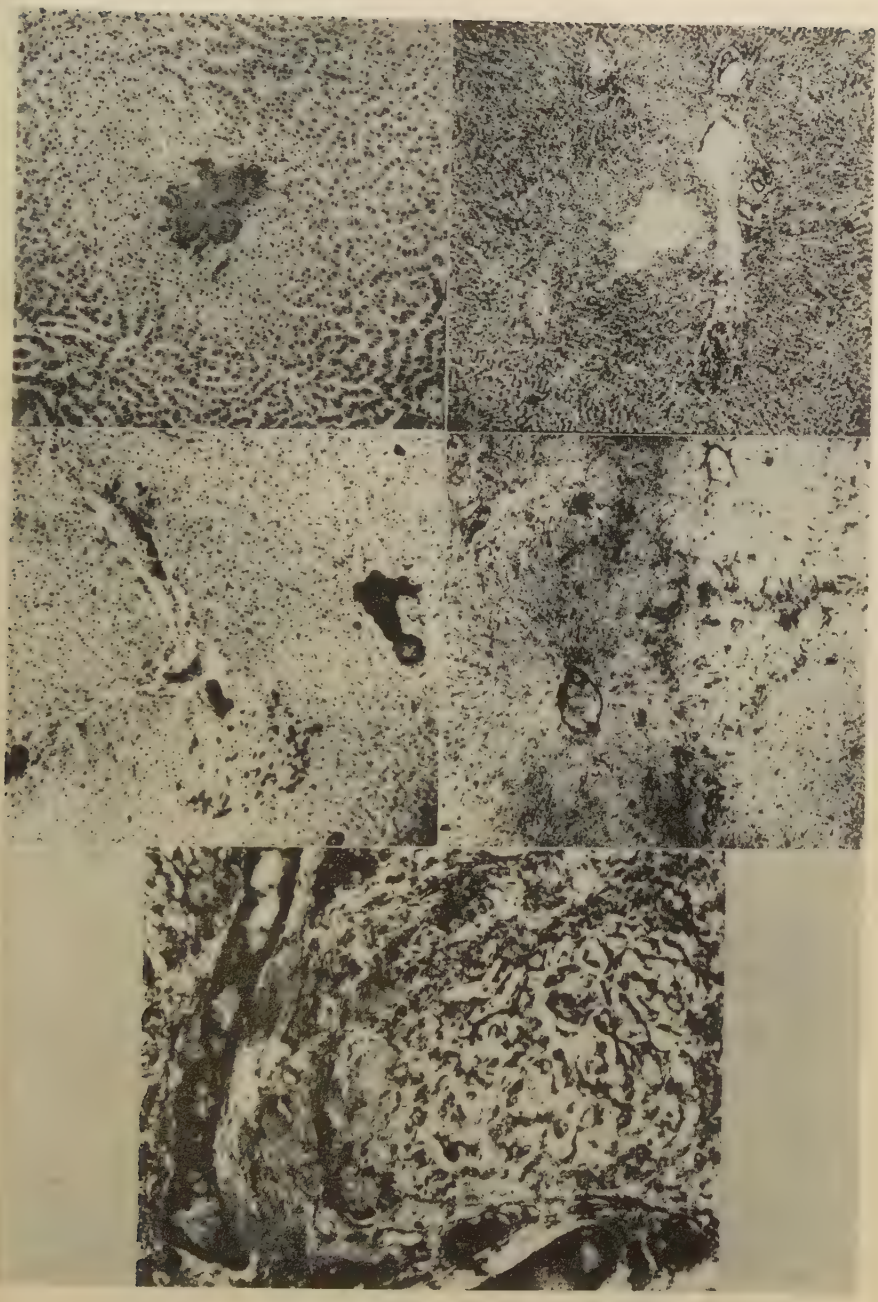

Fig 24

Fig. 25

Fig. 26

Fig. 27

Fig. 28

Fig. 30

Fig. 31

Fig. 32

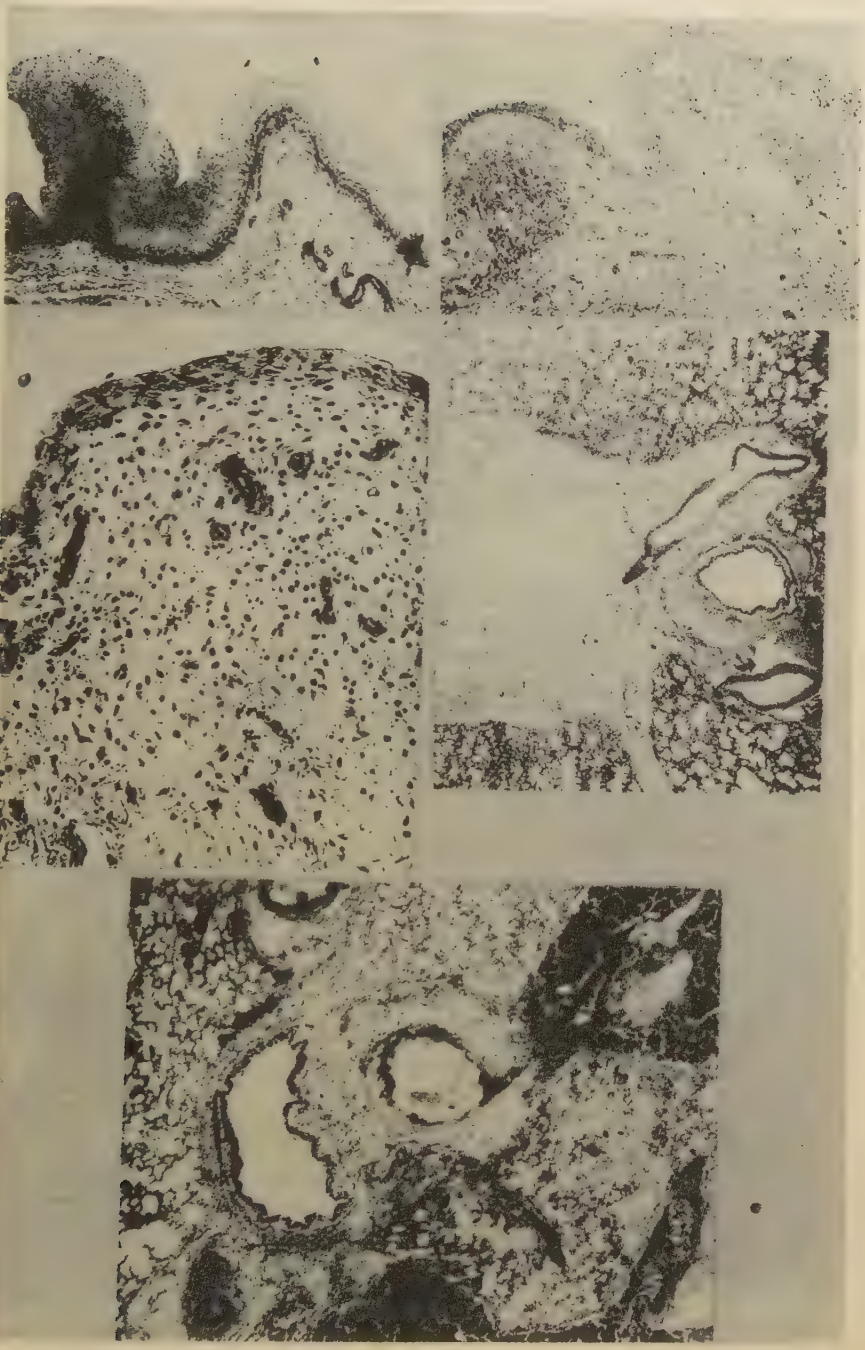

Fig. 34

Fig. 35

spontaneous recovery is said to occur. It may be important to note that McLauchlan removed his animals from further access to bracken. However, since McLauchlan gave the nicotinamide by mouth, his and our results are not exactly comparable. The possibility still remains that nicotinamide given by mouth may influence the rumen micro-organisms in some way.

The cause of the bone marrow lesion remains obscure. Two hypotheses may be suggested.

(1) Depletion hypothesis. The ruminant is largely dependent for its nutrition on its intestinal micro-organisms. As a result of eating bracken the micro-organisms in the rumen are unable to produce a nutrient or nutrients necessary for the integrity of the bone marrow. From the experiments described in this paper and those of other workers it is apparent that this nutrient(s) is not one of the common B vitamins, but the possibility still remains that there is lack of an essential amino-acid or an unknown or yet untried vitamin.

(2) Poisoning hypothesis. The bone marrow may be damaged by a poison either (a) in the bracken itself, or (b) formed by the micro-organisms. Repeated search has failed to reveal any poison in the bracken (review by Boddie, 1947). The only substance so far discovered with likely noxious properties is thiaminase. It is possible that under vitamin B_1 deficient conditions in the rumen the micro-organisms produce a poison as a result of altered metabolism or that they produce a poison from a substrate present in the bracken, or that the breakdown products of vitamin B_1 are themselves poisonous. The answers to these speculations can only be obtained by futher experiments.

CONCLUSIONS

In two experiments, eleven heifers were fed experimentally on bracken (*Pteris aquilina*). In experiment 1, sun dried bracken was fed as 60 per cent, by weight, of the ration with 40 per cent hay or 20 per cent hay and 20 per cent molasses. In experiment 2, sun dried bracken fed as 60 per cent. of the ration for 9 weeks was followed by a diet of fresh bracken only.

All eleven heifers developed overt signs of bracken poisoning in 32 to 86 days. The disease was characterised by widespread haemorrhage and a rise in temperature up to 108°F. (normal 102°F.).

The essential lesion was bone marrow damage, affecting the megakaryocytes, the primitive white cells and the primitive red cells. Thrombocytopenia and leucopenia resulted. The red blood cells in the peripheral blood did not decrease in number per c.mm. until severe bleeding, but the haemoglobin per cent. showed a downward trend.

Terminally concomitant with the rise in temperature there was bacterial invasion of the blood. Bacterial infarction of the liver, lungs and kidney may result.

The subcutaneous administration of vitamin B_1 either alone or together with riboflavin, Ca pantothenate, pyridoxin, folic acid, nicotinamide, biotin and inositol from the beginning of bracken feeding and throughout the experiment did not influence the course of the disease. Unlike the horse and the rat, there was no evidence that cattle fed on bracken suffer from vitamin B_1 deficiency.

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CAPTIONS FOR FIGURES

Fig. 1. Rectal temperature (°F.) of heifers nos. 1, 2 and 3 given 60 per cent. dried bracken in ration till end of 9th week of experiment and thereafter fresh green bracken only, and of heifer no. 4 fed on grass and hay. Note rise in rectal temperature co-incident with bacteraemia*. Experiment 2.
Fig. 2. Average amount of food eaten (lbs./week) by heifers nos. 1, 2, 3 and 4. Amount of food eaten showed greater variation when fresh green bracken was fed. Experiment 2.
Fig. 3. Weekly weight (cwts) of heifers nos. 1, 2, 3 and 4. Experiment 2.
Fig. 4. Platelets X 10^5 per cm.m. of blood, of heifers nos. 1, 2 and 3 (bracken-fed) and of heifer no. 4 (control). Experiment 2.
Fig. 5. Total white cells X 10^3 per cm.m. of blood of heifers nos. 1, 2, 3 and 4. Experiment 2.
Fig. 6. Red blood cells X 10^6 per cm.m. of heifers nos. 1, 2, 3 and 4. Experiment 2.
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Fig. 19. Small intestine. Longitudinal section. Inflammatory reaction around small artery and vein in subserosa. Heifer no. 87121, expt. 1. H. & E. X 100.
Fig. 20. Rumen. Capillary in submucosa. Note swollen endothelial cells, and inflammatory cells around capillary. Heifer no. 3, expt. 2. H. & E. X 825.
Fig. 21. Liver centrilobular necrosis. Heifer no. 66N, experiment 1. There was widespread centrilobular necrosis in this liver. While most of the lobules only show centrilobular necrosis, this section shows in addition a mass of gram negative bacilli which are not causing infarction. H. & E. X 80.

Fig. 22. Liver. Focal necrosis near a portal triad. Heifer no. 1, experiment 2. H. & E. X 60.

Fig. 23. Infarct in liver. Masses of bacteria are present in portal veins and in sinusoids. Heifer no. 1, experiment 2. H. & E. X 60.

Fig. 24. Infarct in liver, heifer no. 22723, experiment 1. Masses of bacteria and fibrin in sinusoids and in central vein. The bacteral masses and fibrin seem to form a ring thus blocking off an area of liver. Gram. X 40.

Fig. 25. Infarct in liver. Area within the infarct. Heifer no. 27723, experiment 1. Note necrotic liver cells; dilated sinusoids; masses of fibrin and bacteria in portal vein at left. Heidenhain's azan stain. X 95.

Fig. 26. Kidney : high power view of infarct shown in Fig. 13. Infarcted area to right showing necrosis of kidney tissue and bacterial masses. At left is a large capillary filled with bacteria. Heifer no. 22749; experiment 1. H. & E. X 30.

Fig. 27. Kidney : heifer no. 22749, experiment 1. Note bacterial masses, grey in colour, filling the peritubular capillaries. The tubules are necrotic. Red blood cells appear black. Heidenhain's azan stain. X 250.

Fig. 28. Kidney : heifer no. 22749, experiment 1. Gram positive bacteria within Bowman's capsule in periphery of infarcted area. H. & E. X 220.

Fig. 29. Kidney : eosinophilic material within Bowman's capsule in a kidney free from bacterial infarction. Heifer no. 22723, experiment 1. H. & E. X 220.

Fig. 30. Kidney : perivascular infiltration with small round cells. Heifer no. 66N, experiment 1. H. & E. X 120.

Fig. 31. Normal bladder from heifer no. 4, experiment 2. For comparison with Fig. 30. Note stratification of epithelial layers and thickness of submucosa. H. & E. X 60.

Fig. 32. Bladder from heifer no. 3, experiment 2. Vacuolation of epithelial cells, and desquamation; haemorrhage into submucosa, and oedema of submucosa. H. & E. X 60.

Fig. 33. Bladder from heifer no. 22749, experiment 1; from above downwards, desquamated epithelial layer, oedematous submucosa with cellular infiltration. H. & E. X 150.

Fig. 34. Lung showing oedema of interseptal space. Heifer no. 22726, experiment 1. H. & E. X 26.

Fig. 36. Lung : haemorrhage into septa; bacteria in masses in vessels. H. & E. X 26. Heifer no. 22726, experiment 1.

EXPERIMENTAL BRACKEN POISONING IN CALVES

By

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INTRODUCTION

This study was undertaken to determine whether young calves fed experimentally on bracken (*Pteris aquilina*) developed a syndrome similar to that of the adult animal suffering from bracken poisoning The use of young animals in seeking for a nutritional deficiency or a toxin or poison, or for testing the effects of a poison, is a well established procedure.

The disease in the adult has now been well described (Storrar, 1893; Stockman, 1917; Boddie, 1947; Naftalin and Cushnie, 1951) and a detailed account of the pathology has been given (Naftalin and Cushnie, 1954). The essential lesion in adult cattle is severe bone marrow damage affecting the primitive white and primitive red cells and the megakaryocytes. In the peripheral blood there is thrombocytopenia and leukopenia; there is little or no change in the red blood cells except for a terminal reduction in numbers associated with severe internal haemorrhage. Bacteraemia is associated with complete, or almost complete, disappearance of polymorphs from the peripheral blood. A rise in temperature up to 107°F. and severe haemorrhage then results. If, after the onset of the bacteraemia, the animal lives some days, bacterial infarction of the liver, lung and kidney may take place. The animal dies from severe internal haemorrhage. The course of the illness in the calves now reported was similar to that found in the adult animals if the young animals lived long enough.

EXPERIMENTAL PROCEDURES

Animals and feeding. Six calves were studied. They were aged between 62 and 105 days, when first offered bracken (Table 1). The calves were reared on milk (5 to 8 pints/day), Calf starter gruel* (6 ozs. to 1 lb./day), oats and linseed cake mixture (1 to 2 lb./day) and fresh grass or hay *ad lib.* The milk was omitted after about the 8th week of age. When the bracken was introduced into the diet the gruel and oats-linseed mixture was omitted. The grass or hay was withdrawn during the first 5 to 10 days of bracken feeding so that the calves finally ate a diet wholly of bracken.

Bracken feeding. Calf 1 (Red-Poll heifer, aged 91 days, weight 160 lb.) served as a control and was fed on fresh grass. (June-July, 1952).

Calves 2 (Red-Poll heifer, aged 91 days, weight 163 lb.) and 3 (Fresian heifer, aged 105 days, weight 182 lb.) were fed on fresh green bracken, cut every other day (June-July, 1952).

Calves 4 and 5 (Ayrshire bulls, aged 62 days, weight 141 lb. and 152 lb.) received 1 lb. of fresh bracken morning and evening and sun-dried

* Calf starter gruel, manufactured by the Caledonian Milling Co., Aberdeen, contains dried whey, dried skim milk, linseed cake, oats, bran, fish meal, minerals, and cod liver oil.

bracken to appetite. The dried bracken harvested in July was chopped in a hay cutter immediately before feeding. From the beginning of the experiment, calf 4 received by drench 250 mg. vitamin B_1 in 150 ml. water at 9 a.m. and at 4.30 p.m. The observations on these two calves were made in September-October, 1952.

Calf 6 (Ayrshire bull, aged 79 days, weight 139 lb.) was fed on dried bracken only (December 1952 - January 1953). Since it was intended to make other studies on this calf, it was fitted with an ebonite rumen cannula during its eighth week of life but as the calf died early in the experiment these were not done.

The food eaten was measured daily about 10 a.m. (Table 1).

Calves 1, 2 and 3 were weighed daily; calves 4, 5 and 6 less frequently. Observations of the heart rate and the rectal temperature were made daily at about 10 a.m.

Haematological observations were made once, twice or three times a week on venous blood. The methods used were those described in the previous experiments (Naftalin and Cushnie, 1954).

Bone marrow biopsy. At the beginning of the experiment on the 14th and 22nd days portions of sternal bone marrow were removed from calf 6 as follows. The skin and deep tissues over the distal sternum were anaesthetised with 2 c.c. of 2 per cent "Planocain". With a 10 c.c. sterile syringe attached to give better purchase, a 14 s.w.g. serum needle with its stylette in place was then pushed into the marrow cavity. The syringe was removed, and by grasping the protuding end with a small pair of sterile forceps, the stylette pushed beyond the tip of the needle. This procedure freed the needle of any plug of bone. The stylette was removed, the syringe again attached and about 1 c.c. of a mixture of bone marrow and blood withdrawn. This mixture was blown on to a watch-glass. Smears were made from marrow flecks which had been picked up by a Pasteur pipette. The remainder of the material was fixed in 10 per cent. formol saline for the preparation of histological sections.

Blood culture. In the terminal stage of the disease, when the temperature rose above 102°F., blood was withdrawn from the jugular vein for bacteriological culture.

RESULTS

Clinical Condition

Towards the end of the experiment calves 2 and 3 lost condition and their hair became rough and staring. Flesh was lost particularly from the hindquarters. Calves 4, 5 and 6 did not show any marked loss of condition, but calf 6 deteriorated rapidly in its last week of life.

Signs typical of bracken poisoning in the adult appeared in calves 2, 3 and 5. Haemorrhage from the nose, and blood in the faeces were observed in calf 3 during the 5th week. In calves 2, 3 and 5 the temperature rose to about 107° to 108°F. on the 29th, 37th and 35th days of eating bracken. The next day calves 2 and 3 had severe haematuria. This was not observed in calf 5 since it was killed on the day its rectal temperature rose to 108°F. (October 13th).

Weight (Table 1)

Calf 1 fed on grass increased in weight from 160 lb. when 91 days old to 203 lb. at 120 days, a gain of 1½ lb. a day. When fed on bracken, calf 2 maintained its weight at about 160 lb., while calf 3 gained slowly from 182 to 202 lb. in 30 days (2/3rd lb. a day) and thereafter in the last 7 days of its life lost 14 lb. (2 lb. a day) Calf 4 maintained its weight at 140 lb.; calf 5 lost 10 lb. in the first week of bracken feeding and thereafter maintained its weight at 142 lb. Calf 6 maintained its weight about 139 lb. for about 7 days, then lost 7½ lb. in the next week and then 22½ lb. in its last week of life, i.e., approximately 3 lb. a day.

TABLE 1

AGE, WEIGHT AND AMOUNT OF BRACKEN EATEN

Calves nos. 1, 2, 3, 4, 5 and 6

Calf no.	*Breed and sex*	*Age (days) at beginning of experiment*	*Weight (lb.) at*		*Average daily consumption of bracken (lb.)*		*Remarks*
			Beginning of experiment	*End of experiment*	*Fresh*	*Dried*	
1	Red-Poll F	91	160	203	..	..	Control calf fed on grass.
2	Red-Poll F	91	163	159	8	..	Typical bracken poisoning. Temp. 107°F. on 29th day. Killed on 30th day.
3	Fresian F	105	182	189	11.2	..	Typical bracken poisoning. Temp. 107°F. on 37th day. Killed on 38th day.
4	Ayrshire M	62	141	141	2	1.5	Died suddenly from heart failure on 29th day. Temp. 102°F. Calf was given by drench 250 mg. vitamin B_1 twice a day from beginning of experiment.
5	Ayrshire M	62	152	144	2	1.6	Typical bracken poisoning. Temp. 108°F. on 35th day. Killed on 35th day.
6	Ayrshire M	79	139	110	..	2.6	Died suddenly from heart failure on 23rd day. Temp. 102°F.

Food Eaten (Table 1)

All five calves fed on bracken ate the ration with apparent distaste. The appetite was maintained till the end of life even when weight was being lost.

TABLE II

TOTAL AND DIFFERENTIAL COUNTS

(peripheral blood)

CALF 1 (normal)

Date	*12/6/52*	*14/6*	*17/6*	*19/6*	*21/6*	*26/6*	*1/7*	*4/7*	*8/7*	*11/7*	*15/7*
Hb % Haldane ..	79	75	81	79	78	73	79	82	75	79	76
RBC 10^6/c.mm. ..	10.07	9.50	10.20	10.18	10.61	10.06	10.40	10.71	10.07	10.20	9.95
WBC 10^3/c.mm. ..	7.00	8.45	8.35	8.70	7.90	10.15	8.85	9.70	12.40	14.05	14.40
Platelets 10^5c.mm. ..	6.80	7.50	8.12	7.84	6.90	5.65	7.10	6.05	*	*	*
Differential leucocyte count %											
Polymorphs ..	12	13	11	15	6	7	15	5	18	17	18
Metamyelocytes ..	..	..	..	..	1	..	..	1	..	..	..
Eosinophils ..	..	..	..	3	1	3	1	1	1	2	4
Basophils ..	1	1	1	1	..	..	..	..	..	..	..
Monocytes ..	13	10	5	11	9	10	10	10	8	6	2
Lymphocytes ..	74	76	83	70	83	80	74	83	73	75	76

↓ *CALF 2* (fed on bracken ↓)

Date	*12/6/52*	*14/6*	*17/6*	*19/6*	*21/6*	*24/6*	*26/6*	*28/6*	*1/7*	*4/7*	*8/7*	*11/7*	*15/7*	*17/7*
Hb % Haldane	75	73	75	74	68	73	78	78	73	76	80	79	75	75
RBC 10^6/c.mm.	10.15	10.25	8.28	9.02	7.89	8.82	9.64	7.90	8.80	9.29	9.90	9.21	9.00	8.62
WBC 10^3/c.mm.	11.05	13.40	11.05	9.20	8.00	10.00	9.90	9.75	9.35	6.95	6.40	5.60	2.80	3.00
Platelets 10^5/c.mm.	4.95	4.65	4.90	3.56	4.55	5.70	3.80	4.00	4.48	4.76	3.02	*	0.50	0.60
Differential leucocyte count %														
Polymorphs	27	33	11	17	15	8	4	15	24	8	15	14	..	..
Metamyelocytes	..	..	..	..	..	..	2	..	..	..	..	..	..	..
Eosinophils	..	2	2	1	1	..	..	1	..	1	3	3	..	..
Basophils	2	1	1	2	..	1	1	..	..	..	..	..	..	..
Monocytes	10	7	12	10	15	5	11	9	11	5	2	..	..	..
Lymphocytes	61	55	74	70	69	86	82	75	65	86	70	83	100	100

Date	*9/6/52*	*10/6*	*12/6*	*14/6*	*17/6*	*19/6*	*21/6*	*24/6*	*26/6*	*28/6*	*1/7*	*4/7*	*8/7*	*11/7*	*15/7*	*10/7*
Hb % Haldane	84	88	87	89	85	85	82	81	84	86	84	87	96	86	80	62
RBC 10^6/c.mm.	10.5	10.65	10.25	10.95	10.69	10.56	10.23	10.10	9.96	10.76	10.55	10.47	11.70	11.85	9.50	7.87
WBC 10^3/c.mm.	9.1	12.35	13.05	10.60	13.00	12.30	12.90	12.40	14.20	11.65	8.10	5.50	6.35	4.35	1.24	1.20
Platelets 10^5/c.mm.	5.49	7.05	8.30	7.90	4.52	6.66	8.90	6.35	5.05	4.20	3.25	2.70	*	*	0.32	0.40
Differential leucocyte count %																
Polymorphs	39	36	32	35	35	31	36	30	34	23	32	38	19	11	3	2
Metamyelocytes	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	..
Eosinophils	1	..	1	2	..	1	2	1	..	5	2	1	1	1	..	..
Basophils	..	..	..	..	..	1	1	1	..	..	..	..	1	..	..	..
Monocytes	10	10	11	8	8	13	10	8	5	4	7	2	2	..	..	..
Lymphocytes	50	54	56	55	57	54	51	60	61	68	65	59	77	88	97	98

CALF 4

↓ (fed on bracken ↓ and given 250 mg. vitamin B_1 twice a day by drench)

Date	*9/9/52*	*12/9*	*15/9*	*18/9*	*23/9*	*26/9*	*30/9*	*3/10*
Hb % Haldane	78	70	76	75	76	78	82	76
RBC 10^6/c.mm.	8.72	7.86	7.80	7.92	9.02	8.26	8.92	8.92
WBC 10^3/c.mm.	9.80	9.20	9.10	8.40	5.15	5.80	3.80	2.85
Platelets 10^5/c.mm.	5.00	7.66	6.00	6.41	4.64	3.62	1.52	0.65
Differential leucocyte count %								
Polymorphs	26	29	26	26	14	16	13	9
Metamyelocytes	..	..	..	..	..	..	..	..
Eosinophils	..	1	2	1	..	..	..	..
Basophils	..	..	..	..	1	1	1	1
Monocytes	14	2	6	4	11	6	8	..
Lymphocytes	60	68	66	69	74	77	78	90

CALF 5 (fed on bracken ↓)

Date	*9/9/52*	*12/9*	*15/9*	*18/9*	*23/9*	*26/9*	*30/9*	*3/10*	*7/10*	*9/10*	*11/10*	*12/10*	*13/10*
Hb % Haldane	..	79	78	80	80	82	88	83	90	88	88	88	90	106
RBC 10^6/c.mm.	..	9.42	8.90	9.06	8.96	9.12	10.12	10.22	9.96	10.39	10.52	10.46	11.76	11.24
WBC 10^3/c.mm.	..	5.15	8.10	8.40	6.85	5.40	4.45	3.70	3.10	3.35	2.25	1.20	1.10	0.70
Platelets 10^5/c.mm.	..	5.82	7.14	5.42	5.95	6.02	4.18	2.53	1.66	1.96	0.89	0.64	0.73	0.56
Differential leucocyte count %														
Polymorphs	..	26	26	29	27	34	30	21	25	10	1	..	..	..
Metamyelocytes	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Eosinophils	..	1	..	..	..	..	..	1	..	1	..	..	..	..
Basophils	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Monocytes	..	12	5	6	11	7	2	5	..	1	1	..	..	..
Lymphocytes	..	61	69	65	62	59	68	73	75	88	98	100	100	100

* No observations made.

[*CALF 6 Overleaf*]

Table II—*cont.*

CALF 6 (fed on bracken on 16/12/52)

Date	*15/12/52*	*23/12*	*30/12*	*6/1/53*	*7/1*
Hb % Haldane ..	70	77	83	88	103
RBC 10^6/c.mm. ..	8.36	9.4	9.6	10.72	12.18
WBC 10^3/c.mm. ..	11.15	8.45	11.45	15.80	12.70
Platelets 10^5/c.mm ..	0.05	9.24	8.22	3.88	2.63
Differential leucocyte count %					
Polymorphs ..	34	27	56	80	77
Metamyelocytes ..	..	..	1	..	1
Eosinophiles ..	..	..	..	..	..
Basophils ..	..	..	..	..	..
Monocytes ..	9	7	3	..	1
Lymphocytes ..	57	66	40	20	20

Heart Rates (Fig. 1)

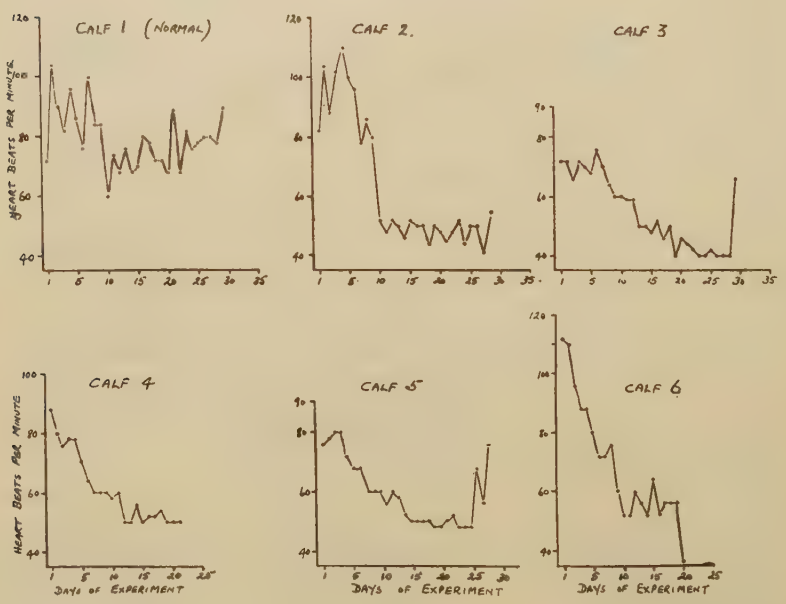
Within 10 days of the beginning of the experiment, the heart rate in calves 2, 4, 5 and 6 decreased from initial rates of 70 to 100 beats a minute to 45 to 55 beats a minute. This change thus began before a diet wholly of bracken was being fed. The bradycardia was uninfluenced by the administration by drench twice daily of 250 mg. vitamin B_1 (calf 4). Calf 3 which was older and larger did not have bradycardia till the beginning of the third week.

When the rectal temperature rose terminally to about 107°F. the heart rates increased up to 75 beats a minute.

Haematology (Table 2)

White cells. In calves 2, 4 and 5 the number of white blood cells per c.mm. in the venous blood was lowered after 14 to 21 days of

Fig. 1



bracken feeding. This suggested that the bone marrow was damaged even earlier. Calf 3 did not show any reduction till 25 to 29 days of bracken feeding. During the three weeks it ate bracken, calf 6 did not exhibit any fall in the total number of white cells per c.mm. in its peripheral blood, but an inversion of the numbers of polymorphs and lymphocytes occurred. The absolute number of polymorphs at the beginning of the experiment was 3,790 per c.mm., the day before death 12,640, and on the day of its death 9,780. In contrast the lymphocytes fell from 6,355 to 3,160, and were 2,540 per c.mm. on the day of its death.

Platelets. In calves 2, 3, 4 and 5, the platelets were much reduced in numbers during the 3rd or 4th week. No remissions were observed. Despite low platelet counts of about 65,000, bleeding did not occur in calf 4. Bleeding was only minimal in calves 2, 3 and 5 till the onset of bacteraemia. These findings have been discussed previously (Naftalin and Cushnie, 1951). Calf 6 died without showing marked thrombocytopenia.

Red cells and Hb. Throughout the experiment there was no change in the r.b.cs. and Hb in calves 2, 3 and 4. Calves 5 and 6 had an increase in r.b.cs. and Hb. In calf 5, r.b.cs. increased from 9.42 million per c.mm. to 11.24 and Hb from 79 to 106 per cent Haldane, and in calf 6, r.b.cs. increased from 8.36 million per c.mm. to 12.18 and Hb from 70 to 103 per cent Haldane (Table 2).

Bone Marrow Biopsies from Calf. No. 6 (Table 3)

The cell distribution on 16/12/52 at the beginning of the experiment fell within normal limits with a myeloid-erythroid ratio of 0.67 (Naftalin and Cushnie, 1954). Histological sections of the marrow fragments revealed normal cellularity.

On the 14th day of eating bracken, the red cell precursors were much reduced relatively to the white cells (myeloid-erythroid ratio 7.69). Histologically the marrow flecks appeared less cellular in all elements. There was a marked decrease in the precursors of the red cells.

One day before death marrow biopsy revealed further changes. The red cell precursors were very much reduced (myeloid-erythroid ratio 52.75) No myeloblasts were observed. There was a great increase in the percentage of the mature polymorphs which now formed 68.4 per cent of the total cells in the smears. Lymphocytes were increased to 13.4 per cent. Histological examination of the marrow flecks revealed an oedematous marrow with very few areas of haematopoetic tissue. The predominant cells were polymorphs and reticulum cells. Megakaryocytes with bizarre nuclei were occasionally seen. The flecks were surrounded with many r.b.cs. and the polymorphs may have been associated with the blood rather than the marrow itself.

TABLE 3

DIFFERENTIAL COUNTS PER CENT.

Bone marrow cells from sternal biopsies of calf no. 6

	16 Dec. 1952 (beginning of expt.)	*29 Dec. 1952 (on 14th day)*	*6 Jan. 1953 (on 22nd day)*
Haemocytoblasts ..	1.0	0.2	0.2
Normoblasts			
Early ..	3.4	0.4	0.4
Intermediate ..	23.4	4.2	0.6
Late ..	26.4	5.2	0.4
Blast cells ..	2.0	1.2	0.0
Pro-myelocytes ..	0.6	2.0	0.4
Myelocytes (Neutrophilic)	3.4	5.0	1.4
Metamyelocytes (Neutrophilic)	4.6	15.8	10.0
Polymorphs (Neutrophilic)	22.4	49.0	68.4
Myelocytes (Eosinophilic)	0.8	0.4	0.2
Metamyelocytes (Eosinophilic)	1.0	2.6	1.8
Eosinophils	2.0	3.4	2.2
Myelocytes (Basophilic)	0.0	0.0	0.0
Metamyelocytes (Basophilic)	0.0	0.0	0.0
Basophils ..	0.0	0.2	0.0
Lymphocytes ..	7.4	8.6	13.4
Monocytes ..	1.0	1.2	0.2
Plasma cells ..	0.2	0.0	0.4
Megakaryocytes ..	0.4	0.6	0.0
Myeloid/erythroid ratio	0.67	7.96	52.75

1000 cells counted on each occasion.

Bacteriology

Blood cultures yielded no growth of organisms till the temperature rose; thereafter gram positive bacilli and gram positive cocci calves 1 and 2) or gram negative bacilli (calf 5) were isolated. Cultures from the liver, spleen and kidneys post mortem of all 5 calves yielded no growth of organisms.

Deaths. Calves 4 and 6 died without showing the haemorrhages and terminal rise in temperature typical of bracken poisoning in the adult animal. Calf 4 appeared well when fed at 7 a.m. on 7th October (29th day). When next observed at 8 a.m. it was dead. On January 6th, the twenty-second day of eating bracken, calf 6 appeared listless. Its heart rate had dropped to 55 beats per minute. The next day it was found lying down and unable to stand. The heart rate was slow—36 beats per minute. It died at 2.30 p.m. Neither of these calves showed any external oedema, haemorrhage or rise in temperature.

Calves 2, 3 and 5 were killed by 60 cc. of nembutal given intravenously.

Post-mortem Examinations

Calves 2, 3 and 5 presented in the main the features of bracken poisoning already described in the adult animal (Naftalin and

Cushnie, 1951, 1954). There were multiple haemorrhages throughout the carcase and viscera. The bone marrow was pale. No infarcts were observed in the liver, kidneys or lungs. Ulceration of the gut was less severe than that previously observed in the adult, but it should be noted that these calves were killed on the day of the rise of temperature or the day after, i.e., at an earlier stage. Presumably these three calves would have died from haemorrhage if the disease had been allowed to progress.

Calves 4 and 6. The most striking lesion in these two calves was dilatation of the heart, particularly of the right side. Calf 4 had a few petechial haemorrhages on the surface of the ventricles; no haemorrhages were observed on the heart of calf 6. The musculature of the heart of calf 6 was soft, and there was a band of firm gelatinous tissue ½ in width and ½ in depth in the auriculo-ventricular groove.

The other viscera in both calves were very congested. The lungs were oedematous; calf 4 had small patches of oedema in the omasal-abomasal junction, on the peritoneal surface of the rumen, in the portal tract and in the bladder. It had in addition a few small pin-point haemorrhages in the parietal pleura, in the capsule of the liver and spleen, and in lower lid of the right eye. The sternal bone marrow of both calves was pale. There were no other lesions noted in either calf. The cause of death was heart failure.

Histology

The histological examination of the tissues of calves 2, 3 and 5 revealed lesions of the type associated with thrombocytopenic purpura. In addition to the two lesions already described in the alimentary tract, i.e., (1) haemorrhage in the submucosa followed by ulceration and bacterial invasion, and (2) areas of oedema with or without plasma cell and mononuclear cell infiltration (Naftalin and Cushnie, 1954, Figs. 9, 10 and 17), a third type of lesion was observed in the rumen of calf 2. This lesion consisted of a necrotic area in the epithelium and subepithelial tissue in which were embedded dense clumps of gram positive cocci. There was no haemorrhage (Fig. 2). This lesion appears to have a different pathogenesis from lesion (1) and is probably associated with the agranulocytosis rather than with thrombocytopenia and capillary damage.

In calves 4 and 6 the outstanding feature was intense congestion of the liver, spleen, kidneys and gut. The gelatinous tissue in the auriculo-ventricular space of calf 6 was found to be composed of myxomatous tissue which joined up with the connective tissue of pulmonary and tricuspid valves (Figs. 3 and 4). There was little or no inflammatory reaction. This lesion has not been encountered previously.

Bone marrow

In all five calves fed on bracken the marrow was so devoid of cells that satisfactory marrow smears were not obtained post mortem and

it was not possible to make differential marrow cell counts. Histologically the sternal marrows were aplastic and oedematous but in calf 6 a few scattered areas of haematopoetic tissue were still present. Megakaryocytes, many with bizarre-shaped nuclei, were present in the bone marrow of this calf. The predominant cell in the marrow of calf 6 is the polymorph, a finding which is consistent with the high peripheral white blood count of 12,700 cells per c.mm. just before death (polymorphs=77 per cent).

DISCUSSION

It is apparent that the typical syndrome associated with bracken poisoning in the adult develops in calves fed on bracken, if they live long enough. Stockman (1917) fed a bull-calf, aged 8 months at the beginning of the experiment, on fresh bracken. The calf died in 32 days showing the typical syndrome. Guilhon and Dradillat (1950) examined 3 calves which had eaten bracken and were aged 4, 6 and 7 months at the time of their death. All showed the typical haemorrhages and leucopenia. However two of our younger calves, 4 and 6, aged 62 and 79 days at the beginning of the experiment, died without showing haemorrhage or rise in temperature. The immediate cause of their death was heart failure, but only in calf 6, which died on the 23rd day of eating bracken, was any morphological change observed in the heart. In all calves fed on bracken the heart rate was slowed; the terminal rise in heart rate in calves 2, 3 and 5 was associated with high temperature. This suggests that the reflex arc is still intact, that the sino-auricular node is sensitive to stimuli and that there is no lesion in the conducting system. Although they died of heart failure both calves 4 and 6 had severe bone marrow damage.

The peripheral white blood counts in calves 2, 3, 4 and 5 followed a pattern we have previously described, i.e., a progressive diminution of all white cells, the polymorphs being particularly affected. In calf 6 the white cells in the peripheral blood behaved differently. The increase in the number of the polymorphs per c.mm. in the peripheral blood is to be contrasted with the progressive destruction of the bone marrow. It is also to be contrasted with the fall in the number of lymphocytes in the peripheral blood, a phenomenon which is present in the other calves and which we have found previously to be associated with bracken poisoning in cattle (Naftalin and Cushnie, 1951 and 1954).

We have insufficient data to provide a full interpretation of this phenomenon but it is evident in this calf that except for a lowered platelet count on the 22nd day the peripheral blood picture gave no indication of the severity and nature of the bone marrow damage. There was no evidence of infection in this calf to account for the polymorphonuclear leucocytosis. The skin around the cannula in the rumen remained clean and neither the rectal temperature nor the heart rate increased.

A further point calls for comment. Two calves (5 and 6) had increase in the number of red cells per c.mm. and Hb per cent in their peripheral blood during the course of the experiment while their bone marrow showed progressive destruction of red cell precursors. This would seem to indicate that these two calves had haemoconcentration, but at present no explanation is being offered why this should be so.

Calf 4 which received 250 mg. vitamin B_1 by drench twice a day died 6 days before calf 5 showed a rise in rectal temperature. The administration of these amounts of vitamin B_1 did not prevent bone marrow damage. Calf 4 had a higher initial white cell count of 9,500 per c.mm. compared with 5,500 per c.mm. of calf 5, but its white cell count fell more rapidly, and though from a consideration of the final blood and terminal bone marrow pictures, it is not possible to draw any clear distinction between these two calves,, it may be that vitamin B_1 given in this way had a toxic effect. The thiaminase present in bracken splits vitamin B_1 finally into pyrimidine and thiazole, and it is quite possible that these final products or their intermediaries may interfere directly with the rumen bacteria or after absorption interfere in some way with the metabolism of the bone marrow.

CONCLUSIONS

Five calves aged 62 to 105 days at the beginning of the experiment were fed on either fresh or dried bracken (*Pteris aquilina*). Another calf fed on grass was studied as a control.

In 3 calves aged 62, 91 and 105 days at the beginning of the experiment the syndrome typical of bracken poisoning in adult cattle developed on the 35th, 29th and 37th days of eating bracken.

Two calves aged 62 days and 79 days died suddenly from heart failure on the 29th and 23rd days without rise in temperature or haemorrhage but they also had severe bone marrow damage.

The essential lesion was bone marrow damage. The most primitive cells of the myeloid and erythroid series and the megakaryocytes were principally affected. Once formed, the mature polymorphs seem to be unaffected.

All five calves had marked bradycardia but only in one calf were morphological changes observed in the heart. The bradycardia was not influenced by the administration of 250 mg. vitamin B_1 twice a day by drench.

At present no further explanation than that already given (Naftalin and Cushnie) is offered for these phenomena, and although the evidence seems to point to the presence of a poison it is still not possible to decide whether these lesions in the bone marrow and the heart can be ascribed to this alone or to a nutritional deficiency, or to both.

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CAPTIONS FOR FIGURES

Fig. 1. Heart rates (beat per minute) of calves nos. 1-6. Calf no. 4 was given from the beginning of the experiment 250 mg. of vitamin B_1 twice daily by drench.
Fig. 2. Rumen of calf 2. Ulceration of mucosa and invasion of subepithelial tissue by masses of gram positive bacilli. H & E. X 15.
Fig. 3. Section from fatty tissue in auriculo-ventricular groove. Normal calf. Masson trichrome. X 70.
Fig. 4. Section of tissue from auriculo-ventricular groove of calf no. 6. Note the increased cellularity, increase in fibres and absence of inflammatory reaction. Masson trichrome. X 70.

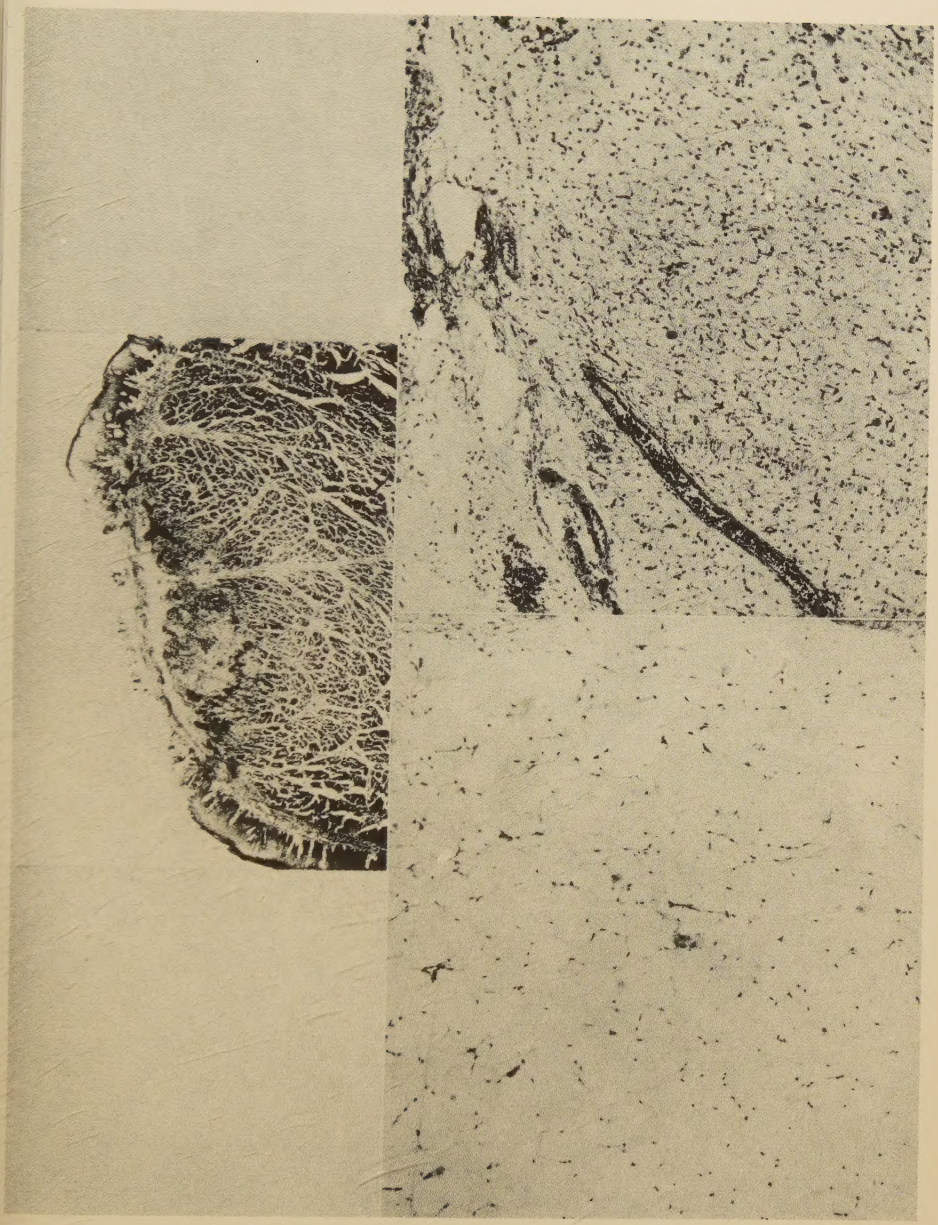

Fig. 4

Fig. 3

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